Cassava Virus Diseases: Biology, Epidemiology, and Management

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Abstract

Cassava (*Manihot esculenta* Crantz.) is the most important vegetatively propagated food staple in Africa and a prominent industrial crop in Latin America and Asia. Its vegetative propagation through stem cuttings has many advantages, but deleteriously it means that pathogens are passed from one generation to the next and can easily accumulate,

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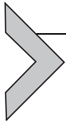
threatening cassava production. Cassava-growing continents are characterized by specific suites of viruses that affect cassava and pose particular threats. Of major concern, causing large and increasing economic impact in Africa and Asia are the cassava mosaic geminiviruses that cause cassava mosaic disease in Africa and Asia and cassava brown streak viruses causing cassava brown streak disease in Africa. Latin America, the center of origin and domestication of the crop, hosts a diverse set of virus species, of which the most economically important give rise to cassava frog skin disease syndrome. Here, we review current knowledge on the biology, epidemiology, and control of the most economically important groups of viruses in relation to both farming and cultural practices. Components of virus control strategies examined include: diagnostics and surveillance, prevention and control of infection using phytosanitation, and control of disease through the breeding and promotion of varieties that inhibit virus replication and/or movement. We highlight areas that need further research attention and conclude by examining the likely future global outlook for virus disease management in cassava.



1. INTRODUCTION

1.1. Cassava: the plant, its cultivation and current economic importance

Cassava (*Manihot esculenta* subspecies *esculenta* Crantz) is a perennial shrub from the family Euphorbiaceae. Its geographical origins remain a topic of research debate, but the most recent evidence based on molecular markers suggests that the plant was domesticated within the southwestern rim of the Amazon basin (in modern day Brazil) and is derived from its closest wild relative, *M. esculenta* ssp. *flabellifolia* (Pohl) (Léotard et al., 2009; Olson & Schaal, 1999). Cassava plants typically reach 1–4 m in height at physiological maturity, and the tuberous roots produced may be harvested from 6 months to 4 years after planting. These roots, which typically have a dry matter content of 30–40%, provide an important source of starch, and in communities in South America that have cultivated the crop for many generations, a wide variety of processed products have been developed. The broad environmental adaptability of cassava and its tolerance of acid soils and sustained periods of drought were key factors in its widespread adoption throughout the tropical Americas. Although monoecious cassava plants produce fertile seeds, and these may be planted, the standard cultivation system makes use of stem cuttings for propagation and establishing a new crop. Vegetative propagation ensures uniformity of a crop variety from season to season and means that planting a new crop is relatively simple; however, this also has the negative consequence of

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sustaining pathogen populations from one cropping cycle to the next, a fact that is particularly significant in the epidemiology of viruses that infect the plant.

Cassava may have been domesticated as much as 10,000 years ago, but it did not spread beyond Latin America until the sixteenth century, when Portuguese traders introduced it to the western Atlantic shores of Africa, in the Gulf of Guinea (Carter, Fresco, Jones, & Fairbairn, 1997). Diffusion inland occurred slowly after that, but by the period of European "exploration" into the interior in the nineteenth century, cassava cultivation had become widespread throughout much of the tropical belt of West, Central, and East Africa. The eighteenth and nineteenth centuries also saw the introduction of cassava to much of south and southeast Asia (Onwueme, 2002). By the start of the twenty-first century, cassava was being widely cultivated throughout the tropics and had become a globally important crop, providing an essential source of carbohydrates to hundreds of millions of people and offering diverse commercial and industrial applications via transformation processes. Although Latin America remains a major producer, more than half of global production is currently in Africa (FAOSTAT, 2014). In spite of its preeminence in overall production of cassava, Africa has lower average yields (10.9 t/ha) than both South America (13.2 t/ha) and Asia (19.7 t/ha) (FAOSTAT, 2014). Much of the production in Asia is grown for either animal feed exports (Thailand and Vietnam) or starch (India). Europe provided the major market for these products in the twentieth century, but rapid growth in demand in recent years means that China is now the main importer of cassava products, importing more than 15 million tons in 2011 (FAOSTAT, 2014).

1.2. Threats to cassava production

Cassava is affected by a diverse set of constraints. Some of the most important of these are pests and diseases. Arguably, the greatest deleterious global impacts on cassava production have resulted from the inadvertent introduction of insect pests or disease-causing pathogens to regions in which they did not previously occur. The most important examples of this have been the introductions of the arthropod pests—cassava mealybug [*Phenacoccus manihoti* Mat.-Ferr.] (CM), cassava green mite [*Mononychellus tanajoa* (Bondar)] (CGM), and cassava bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *manihotis*—to Africa in the 1970s, and the more recent introductions of CM and CBB to southeast Asia (CIAT, 2010). These alien invasive introductions have also been associated with some of the greatest

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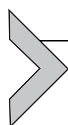
successes in cassava pest and disease management in the form of classical biological control programs that have resulted in dramatic reductions in the incidence and damage caused by CGM in Africa and CM in both Africa and Asia (IITA, 2010a; Neuenschwander, 1994; Yaninek, Onzo, & Ojo, 1993). Relatively less success has been achieved, by contrast, in managing pests and diseases that are indigenous to their respective continents. Most notable among these are the virus diseases. A diverse set of virus species infect cassava in Latin America, although the most economically important of these are the species that give rise to the cassava frogskin disease (CFSD) syndrome. Although this has been recognized for many years (Pineda, Jayasinghe, & Lozano, 1983), there is still uncertainty about the precise etiology, and evidence has been presented for the involvement of phytoplasmas (Alvarez, Mejía, Llano, & Loke, 2009) as well as several virus species (Carvajal-Yepes et al., 2014). There are fewer virus groups affecting cassava in Africa and fewer still in Asia. However, the large and increasing economic impact of cassava mosaic geminiviruses (CMGs) that cause cassava mosaic disease (CMD) in Africa and Asia and cassava brown streak viruses (CBSVs) causing cassava brown streak disease (CBSD) in Africa is such that they are currently considered to be the greatest global threat to cassava production (Legg, Somado, et al., 2014). In this chapter, we examine the biology and epidemiology of the most important groups of viruses infecting cassava in the world, and review control tactics and disease management strategies. In so doing, we highlight areas that need further research attention and conclude by examining the likely future outlook for virus disease management in cassava.



2. BIOLOGY AND EPIDEMIOLOGY OF CASSAVA VIRUSES

2.1. Viruses of cassava

2.1.1 Africa and South Asia

2.1.1.1 Introduction

Following the introduction of cassava to Africa in the sixteenth century, it became infected by a unique set of viruses, none of which are recorded from the crop's center of origin in South America. About 15 virus species and several strains have been identified infecting cassava in Africa and its offshore islands (Table 1). Eleven of these are responsible for the two most devastating diseases, namely: CMD and CBSD. *Cassava green mottle virus*, Cassava virus C and Cassava Kumi virus A & B have been reported infecting cassava but they were not well characterized, and their significance is not known (Table 1).

Table 1 The viruses of cassava

Virus name	Genus/Family	References	Sequence(s) available[a]	Diagnostics[b]	Distribution
(a) Latin America					
Cassava common mosaic virus (CsCMV)	*Alphaflexiviridae/ Potexvirus*	Costa (1940), Silva, Kitajima, and Oliveira (1963), and Kitajima, Wetter, Oliveira, Silva, and Costa (1965)	NC_001658	ELISA/ RT-PCR	Colombia, Brazil (isolated cases from Africa, Asia)
Cassava vein mosaic virus (CsVMV)	*Caulimoviridae/ Cavemovirus*	Costa (1940), de Kochko et al. (1998)	NC_001648	PCR	Brazil
Cassava virus X (CsVX)	*Alphaflexiviridae/ Potexvirus*	Lennon, Aiton, and Harrison (1986)	NA[e]	ELISA/ RT-PCR	Colombia
Cassava new alphaflexivirus (CsNAV)	*Alphaflexiviridae/ Potexvirus*	Carvajal-Yepes et al. (2014)	KC505252	RT-PCR	Colombia
Cassava frogskin-associated virus (CsFSaV)	*Reoviridae/ Oryzavirus*	Calvert, Cuervo, Lozano, Villareal, and Arroyave (2008)	DQ139870	RT-PCR	Colombia, Brazil, Costa Rica, Argentina
Cassava polero-like virus (CsPLV)	*Luteoviridae/ Polerovirus*	Carvajal-Yepes et al. (2014)	KC505249	RT-PCR	Colombia, Costa Rica
Cassava torrado-like virus (CsTLV)	*Secoviridae/ Torradovirus*	Carvajal-Yepes et al. (2014)	KC505250, KC505151	RT-PCR	Colombia, Argentina
Cassava symptomless virus (CsSLV)	*Rhabdoviridae/ Nucleorhabdovirus*[c]	Kitajima and Costa (1979)	NA	NA	Brazil
Cassava Caribbean mosaic virus (CsCaMV)	*Alphaflexiviridae/ Potexvirus*[c]	Lennon et al. (1986)	NA	NA	Colombia
Cassava Colombian symptomless virus (CsCSLV)	*Alphaflexiviridae/ Potexvirus*[c]	Lennon et al. (1986)	NA	NA	Colombia
Cassava American latent virus (CsALV)	*Secoviridae/ Nepovirus*[c]	Walter, Ladeveze, Etienne, and Fuchs (1989)	NA	NA	Brazil, Guyana

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Table 1 The viruses of cassava—cont'd

(b) Africa

Cassava mosaic disease					
African cassava mosaic virus (ACMV)	*Begomovirus*/ *Geminiviridae*	Morris, Coates, Lowe, Richardson, and Eddy (1990)	X17095, X17096	PCR and Real-time PCR	SSA[d]
African cassava mosaic Burkina Faso virus (ACMBFV)	*Begomovirus*/ *Geminivridae*	Tiendrébéogo et al. (2012)	HE616777, HE616778	PCR and Real-time PCR	Burkina Faso
Cassava mosaic Madagascar virus (CMMGV)	*Begomovirus*/ *Geminivridae*	Harimalala et al. (2012)	HE617299, HE617300	PCR and Real-time PCR	Madagascar
East African cassava mosaic Cameroon virus (EACMCV)	*Begomovirus*/ *Geminiviridae*	Fondong et al. (2000)	AF112354, AF112355	PCR and Real-time PCR	SSA and Comoros
East African cassava mosaic Kenya virus (EACMKV)	*Begomovirus*/ *Geminiviridae*	Bull et al. (2006)	AJ717580, AJ704965	PCR and Real-time PCR	East Africa, Madagascar, Seychelles, Comoros
East African cassava mosaic Malawi virus (EACMMV)	*Begomovirus*/ *Geminiviridae*	Zhou, Robinson, and Harrison (1998)	AJ006460, N/A	PCR and Real-time PCR	Malawi
East African cassava mosaic virus (EACMV)	*Begomovirus*/ *Geminiviridae*	Bull et al. (2006)	AJ717542, AJ704949	PCR and Real-time PCR	SSA
East African cassava mosaic virus-Ugandan Variant (EACMV-UG)	*Begomovirus*/ *Geminiviridae*	Pita et al. (2001)	AF126804-7	PCR and Real-time PCR	SSA
East African cassava mosaic Zanzibar virus (EACMZV)	*Begomovirus*/ *Geminiviridae*	Bull et al. (2006)	AJ717562, AJ704942	PCR and Real-time PCR	Zanzibar, Madagascar
South African cassava mosaic virus (SACMV)	*Begomovirus*/ *Geminiviridae*	Berrie, Rybicki, and Rey (2001)	AF155806, AF155807	PCR and Real-time PCR	South Africa, Madagascar, Zimbabwe

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Cassava brown streak disease					
Cassava brown streak virus (CBSV)	*Ipomovirus*/ *Potyviridae*	Winter et al. (2010)	FN434436	RT-PCR, Real-Time RT-PCR, RT-LAMP, and ELISA	Kenya, Mozambique, Uganda, Tanzania, Malawi, Rwanda, Burundi and DR Congo.
Ugandan cassava brown streak virus (UCBSV)	*Ipomovirus*/ *Potyviridae*	Mbanzibwa, Tian, Mukasa, and Valkonen (2009)	FJ039520	RT-PCR, Real-Time RT-PCR, RT-LAMP, and ELISA	Kenya, Mozambique, Uganda, Tanzania, Malawi, Rwanda, Burundi and DR Congo.
(c) South Asia and minor viruses					
Cassava mosaic disease					
Indian cassava mosaic virus (ICMV)	*Begomovirus*/ *Geminiviridae*	Malathi, Nair, and Shantha (1985), Hong, Robinson, and Harrison (1993)	NC_001932, NC_001933	PCR	Southern India and Sri Lanka
Sri Lankan cassava mosaic virus (SLCMV)	*Begomovirus*/ *Geminiviridae*	Saunders et al. (2002)	AJ314737, AJ314738	PCR	Southern India and Sri Lanka
Cassava viruses not linked with any major disease[c]					
Cassava virus C (CsVC) (syn. Cassava Q virus)	*Ourmiavirus*/ Unassigned	Calvert and Thresh (2002), Rastgou et al. (2009)	FJ157981-83	NA[e]	Ivory Coast
Cassava green mottle virus (CsGMV)	*Nepovirus*/ *Comoviridae*	Lennon, Aiton, and Harrison (1987)	NA	NA	Australasia and Pacific Islands, Solomon Islands
Cassava Ivorian bacilliform virus (CIBV)	*Anulavirus*/ *Bromoviridae*	Fargette, Roberts, and Harrison (1991), Scott, MacFarlane, McGavin, and Fargette (2014)	NA	NA	Ivory Coast
Cassava Kumi viruses A and B	Uncharacterized	Calvert and Thresh (2002)	NA	NA	Kumi district of Uganda

[a]GenBank accession numbers of reference isolates of viruses provided.
[b]Many methods are available. Most common and current method(s) indicated.
[c]Limited knowledge on disease biology and causal virus, reliable diagnostic tools are yet to be developed.
[d]SSA, sub-Saharan Africa.
[e]NA, not available.

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Cassava Ivorian bacilliform virus (CIBV) (genus *Anulavirus*; family *Bromoviridae*) has recently had its full genome characterized (Scott et al., 2014) but has only been recorded from Ivory Coast and has no known effect on cassava. This section will consider the two main groups of cassava viruses that cause CMD and CBSD.

2.1.1.2 Cassava mosaic geminiviruses

The causal agent of CMD was initially named as cassava latent virus (Bock, Guthrie, & Figueiredo, 1981) but was subsequently characterized and renamed as *African cassava mosaic virus* (ACMV) (Bock & Woods, 1983; Stanley & Gay, 1983) (genus *Begomovirus*; family *Geminiviridae*). Between 1983 and 2006, seven different Begomovirus species were identified in association with CMD in different regions of Africa (Table 1; Figs. 1 and 2) (Alabi, Kumar, & Naidu, 2011; Legg & Fauquet, 2004; Patil & Fauquet, 2009): ACMV, *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Malawi virus* (EACMMV), *South African cassava mosaic virus* (SACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Zanzibar virus* (EACMZV) and *East African cassava mosaic Kenya virus* (EACMKV). The most recent additions to this list are *Cassava mosaic Madagascar virus* (CMMGV, Harimalala et al., 2012) and *African cassava mosaic Burkina Faso virus* (ACMBFV, Tiendrébéogo et al., 2012). In addition, several strains of these viruses have been identified. The most notable of these is East African cassava mosaic virus-Uganda (EACMV-UG) also known as the "Uganda variant" (Zhou et al., 1997). EACMV-UG was the first recorded example of a begomovirus that has arisen through recombination between two distinct begomovirus species (EACMV and ACMV) (Zhou et al., 1997).

Indian cassava mosaic virus (ICMV) was the first CMG to be recorded from South Asia (Malathi et al., 1985), followed by *Sri Lankan cassava mosaic virus* (SLCMV) several years later (Saunders et al., 2002). Although SLCMV was initially reported from Sri Lanka, it was subsequently shown to occur also in southern India, together with ICMV (Anitha, Makeshkumar, & Edison, 2011; Patil, Rajasubramaniam, Bagchi, & Dasgupta, 2005).

Coinfection with more than one species or strain is a common feature in the etiology of CMD in Africa. Where mixtures are composed of ACMV and one of the EACMV-like CMG species, a synergistic interaction between the species occurs, resulting in an increased overall virus titer leading to more severe symptoms (Fondong et al., 2000; Harrison, Zhou, Otim-Nape, Liu, & Robinson, 1997; Ogbe, Thottappilly, Dixon, & Mignouna, 2003). Rapid regionwide spread of EACMV-UG and ACMV, frequently

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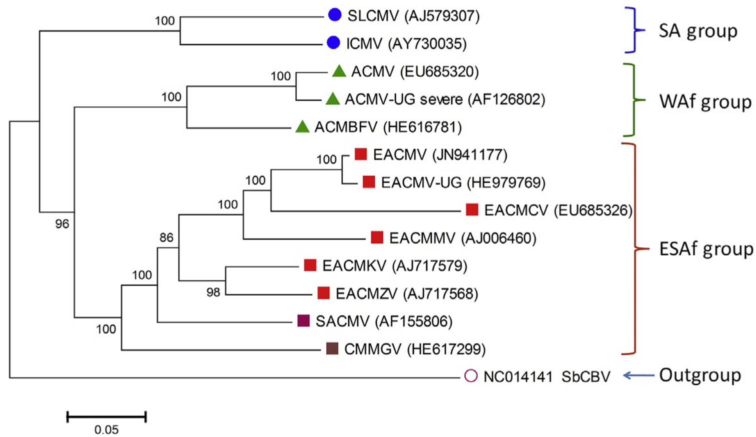

Figure 1 Phylogenetic relationships of the cassava mosaic geminiviruses. Phylogenetic relationship of 11 species of cassava mosaic geminiviruses based on the alignment of complete DNA-A using MEGA 5 software (Tamura et al., 2011). Sequences were aligned using the ClustalW algorithm, and the tree was constructed by the Neighbor-Joining method. The tree was rooted using *Soybean chlorotic blotch virus* (SbCBV) as an outgroup. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown at the branch nodes. There were a total of 2627 positions in the final dataset. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The NCBI GenBank accession numbers of 14 DNA-A sequences are indicated in parenthesis. ACMV: *African cassava mosaic virus*; ACMV-UG: ACMV-Uganda; ACMBFV: *African cassava mosaic Burkina Faso Virus*; CMMGV: *Cassava mosaic Madagascar virus*; EACMCV: *East African cassava mosaic Cameroon virus*; EACMKV: *East African cassava mosaic Kenya virus*; EACMMV: *East African cassava mosaic Malawi virus*; EACMZV: *East African cassava mosaic Zanzibar virus*; EACMV: *East African cassava mosaic virus*; EACMV-UG: EACMV-Uganda; ICMV: *Indian cassava mosaic virus*; SACMV: *South African cassava mosaic virus*; SLCMV: *Sri Lankan cassava mosaic virus*; SA: South Asian; WA: West African; ESA: Eastern and Southern African.

in mixed infections, was an important feature of the African severe CMD pandemic (Harrison et al., 1997; Legg, 1999; Otim-Nape et al., 1997).

The genome of CMGs comprises two circular single-stranded DNA molecules (DNA-A and DNA-B) of about 2.8 kb each, encapsidated in 30 × 20-nm twinned icosahedral particles that replicate by rolling circle amplification through a dsDNA intermediary stage (Hanley-Bowdoin, Settlage, Orozco, Nagar, & Robertson, 1999). DNA-A carries six open reading frames (ORFs), with each encoding a specific protein: AC1, the replication-associated protein (Rep); AC2, the transcriptional activator

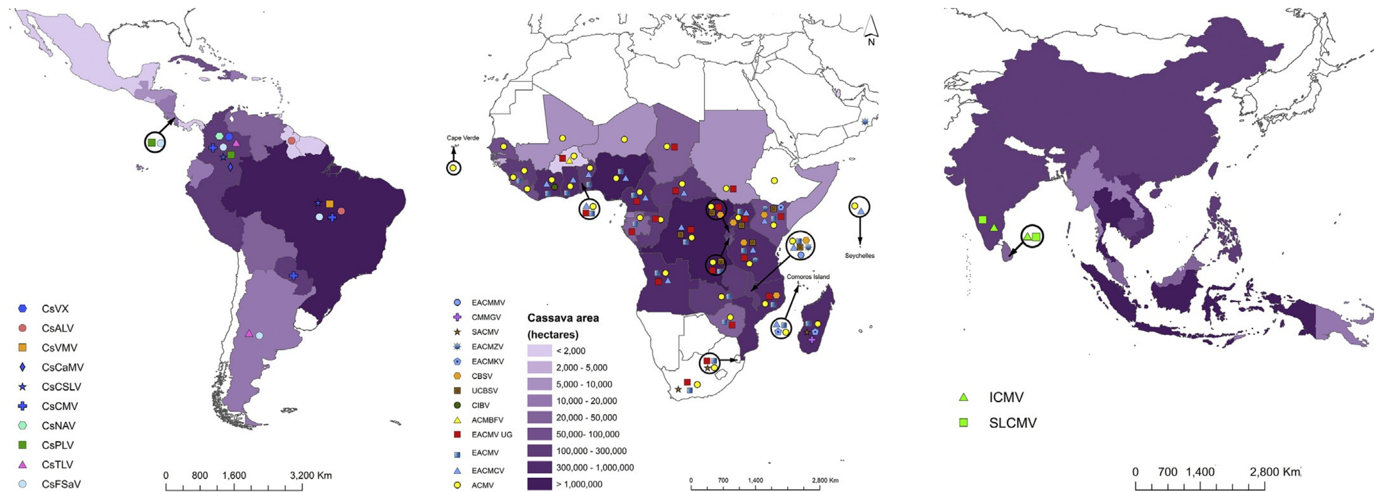

Figure 2 Global distribution of viruses affecting cassava.

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protein (TrAP); AC3, the replication enhancer protein (REn); AC4, the RNA-silencing suppressor; AV1, the coat protein (CP); and AV2, the pre-coat protein. DNA-B has two ORFs: BV1 encodes the nuclear-shuttle protein and BC1 encodes the movement protein (MP).

2.1.1.3 Cassava brown streak viruses

CBSD was first reported in Tanzania by Storey (1936) and was considered to have a viral etiology from the outset. Although several efforts were made to identify the virus causing CBSD during the twentieth century (Bock, 1994a), it was not until early in the twenty-first century that its identity was confirmed (Monger, Seal, Isaac, & Foster, 2001) and the first sequence data provided. Following the characterization of whole genomes of several viruses isolated from CBSD-infected plants, it was shown that two species of CBSVs are involved in the etiology of CBSD: *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) (genus *Ipomovirus*, family *Potyviridae*) (Mbanzibwa et al., 2009; Winter et al., 2010) (Table 1; Figs. 2 and 3).

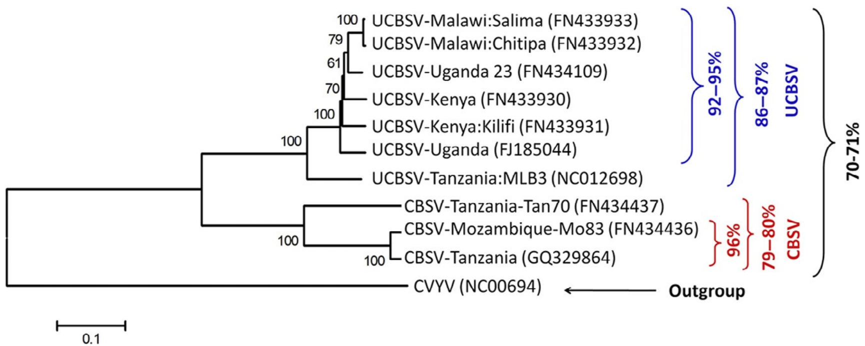

Figure 3 Phylogenetic relationships of the cassava brown streak viruses. Phylogenetic relationship of two cassava brown streak virus species based on the alignment of complete genome sequences using MEGA 5 software (Tamura et al., 2011). Sequences were aligned using the ClustalW algorithm, and the tree was constructed by the Neighbor-Joining method. The tree was rooted by using *Cucumber vein yellows virus* (CVYV) as an outgroup. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown at the branch nodes. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The NCBI GenBank accession numbers of 11 sequences are given in parentheses. Percentage sequence homologies within and between various clusters are indicated. CBSV: *Cassava brown streak virus*; UCBSV: *Ugandan cassava brown streak virus*.

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The complete genomes of these two single-stranded RNA viruses (four CBSV isolates and eight UCBSV isolates, respectively) are 69.0–70.3% and 73.6–74.4% identical at the nucleotide and polyprotein amino acid sequence levels, respectively (Mbanzibwa et al., 2011). The viral genome is expressed as a polyprotein, which is subsequently cleaved by viral proteinases to produce the mature proteins. The genome structures of CBSV and UCBSV are similar, but they differ from other ipomoviruses (Mbanzibwa et al., 2009; Winter et al., 2010). CBSV and UCSBV encode a single P1 proteinase that functions as a suppressor of RNA silencing (Mbanzibwa et al., 2009). Neither of the CBSVs encodes a helper component proteinase (HC-Pro). The most unique feature of the CBSVs is the HAM1h protein, which is a putative nucleoside triphosphate pyrophosphatase, and is situated between the viral replicase (NIb) and the CP in the C-proximal part of the polyprotein. This has only previously been reported for *Euphorbia ringspot virus* (genus *Potyvirus*, family *Potyviridae*) (Mbanzibwa et al., 2009). Based on the analysis of CP genes of CBSV and UCBSV, the two viruses have been shown to be undergoing active but slightly different patterns of evolution (Mbanzibwa et al., 2011).

2.1.2 Latin America

Viruses reported infecting cassava in the "new world" are diverse and belong to virus families *Alphaflexiviridae*, *Reoviridae*, *Secoviridae*, and *Luteoviridae* among the RNA viruses, and to the family *Caulimoviridae* among the DNA viruses (Table 1). Unlike in Africa, no geminivirid or potyvirid sequences have been reported associated with diseases in cassava in the Americas (Calvert, Cuervo, & Lozano, 2012). Recent field surveys in Colombia have detected the common occurrence of *Cassava frogskin-associated virus* (CsFSaV) in fields affected by CFSD, the most economically important disease of cassava in Latin America. CsFSaV is a reovirus of isometric particles of ~70 nm containing a genome consisting of 10 dsRNA segments (Calvert et al., 2008). Such segments may differ in size distribution and distinct dsRNA patterns can be observed in samples collected in the Amazonas region when compared with samples collected in other regions of Colombia. This is confirmed by phylogenetic analyses of the replicase region from different isolates which indicate that the more diverse CsFSaV sequences are found in the Amazonas region (Cuervo, M. et al., unpublished results). Although CsFSaV is associated with the rot symptoms characteristic of CFSD, in single infections it does not induce leaf symptoms in the cassava indicator variety "Secundina" (Carvajal-Yepes et al., 2014; Fig. 4).

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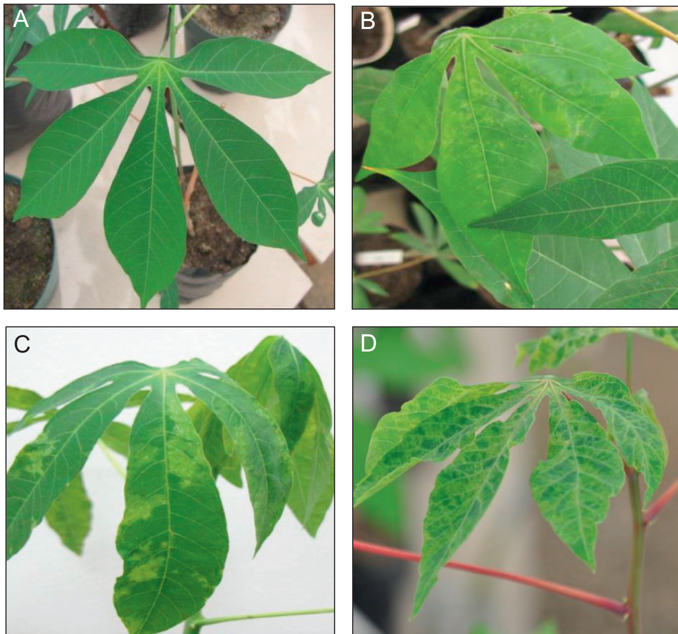

Figure 4 Virus symptoms observed in cassava plants of the landrace Secundina (COL2063) infected by viruses detected in Latin America. Symptoms shown develop around 3 weeks after grafting on noninfected Secundina rootstocks: (A) uninfected control, (B) infected with CsTLV, (C) infected with CsCMV, and (D) mixed infected with CsCMV, CsFSaV and CsTLV. Secundina plants single infected with CsFSaV rarely show symptoms in leaves.

The second-most common virus found in Colombia is Cassava torrado-like virus (CsTLV), a newly described virus species belonging to the *Torradovirus* genus. CsTLV has a bipartite genome of ~10,000 nt and isometric virion particles of ~25 nm (Carvajal-Yepes et al., 2014). Similar particles had been previously observed in preparations of CsFSaV from CFSD-affected plants (Calvert et al., 2008), and the virus has also been detected in frozen samples from symptomatic plants that were collected in the 1980s, suggesting that the virus has been present for longer than previously thought (Carvajal-Yepes et al., 2014). When analyzed at sequence level, a high degree of variability is detected, and even generic primers (Verbeek, Tang, & Ward, 2012) are not able to detect all isolates collected in the same region (Carvajal-Yepes et al., 2014).

Cassava common mosaic virus (CsCMV) (Costa, 1940) has been reported in several countries in South America in association with symptoms of mosaic and chlorosis in leaves (Calvert et al., 2012), although recent

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reexaminations of some of these samples have recorded the presence of additional newly described viruses that may have contributed to the symptoms initially attributed to CsCMV. CsCMV belongs to the *Potexvirus* genus with elongated, semiflexuous particles of 15 × 495 nm (Kitajima et al., 1965). To date, there are only two reported sequences of CsCMV isolates from Brazil, and because they only share ca. 80% nucleotide identity, it seems likely that other distinct strains of the virus exist.

Cassava vein mosaic virus (CsVMV), first reported from Brazil in 1940 (Costa, 1940), is the type species of the genus *Cavemovirus* (family *Caulimoviridae*). It is the only pararetrovirus of cassava, and has so far only been reported from Brazil. Virus particles are quasispherical, 45–50 nm in diameter, and encapsidate a single circular dsDNA genome of ~8159 bp (de Kochko et al., 1998). Virus symptoms in infected plants include chlorosis along the veins, mosaic, and leaf distortion. CsVMV spreads readily through vegetative propagation but transmission through seed or an insect vector has not been detected. There is scant information on the biology, epidemiology, or control of the disease caused. One study suggests that there are nonsignificant differences in yield between CsVMV-infected and uninfected plants (Santos et al., 1995). However, the 35S promoter sequence derived from the CsVMV genome is extensively used as a constitutive promoter in the genetic engineering of plants (Verdaguer et al. 1996).

A diverse range of mechanically transmitted potexviruses have been detected in symptomless cassava, including *Cassava virus X* (CsVX) and Cassava new alphaflexivirus (CsNAV) (Carvajal-Yepes et al., 2014; Harrison, Lennon, & Aiton, 1986). However, because these viruses do not cause symptoms, it is difficult to determine their distribution or to evaluate their importance. In fact, at least four other potexviruses infecting cassava have been reported since the 1960s (Table 1), but for these no sequences or even antisera are available, therefore it is not possible now to identify them to the species level.

2.2. Diseases caused by cassava viruses

2.2.1 Cassava mosaic disease

The first report of viruses affecting cassava was made from East Africa toward the end of the nineteenth century (Warburg, 1894). Here, the term "Kräuselkrankheit" was used to describe the mottled mosaic-like symptoms, leaf deformation, and stunted growth seen in affected plants. The first real evidence for a viral etiology was presented several decades later (Storey, 1938), although this was not to be definitively confirmed and causal viruses named until the development and application of early molecular techniques

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(Bock & Woods, 1983). CMD in India has a more recent described history. The first published record of its presence was during the 1960s (Alagianagalingam & Ramakrishnan, 1966), although this source refers to an earlier report made a decade previously (Abraham, 1956). The earliest reports of CMD in India noted that the disease was restricted to the cassava-growing regions of southern India: primarily Kerala and Tamil Nadu, and to a lesser extent Karnataka and Andhra Pradesh (Malathi et al., 1985). More recently, CMD was also reported from Sri Lanka (Austin, 1986). Although there is variation in severity associated with the mix of species causing CMD in South Asia, in all cases the general symptoms are the same as those observed in Africa, hence the use of a single name to describe the disease, regardless of the CMG species causing the infection (Calvert & Thresh, 2002). CMD has not yet been recorded at any location in South America or Southeast Asia. It is assumed that this is a consequence of the absence of suitable insect vectors in these regions.

2.2.2 Cassava brown streak disease

CBSD was first reported during the early days of the Amani (northeastern Tanzania) cassava research program in the 1930s (Storey, 1936, 1938). At the time of this earliest record, it was already noted that CBSD occurred widely in the coastal region of East Africa. Three major symptom types were recognized: a blotchy yellow chlorosis of mature leaves often associated with minor veins; brown, round, or elongate streak-like lesions on the young green portion of stems; and dry, brown necrotic lesions in the tuberous roots (Storey, 1936). The viral etiology of CBSD was subsequently proved through the demonstration of Koch's postulates (Winter et al., 2010).

Prior to the twenty-first century, the distribution of the disease remained restricted to coastal East Africa—from northeastern Kenya in the north to Mozambique in the south, and inland to the shores of Lake Malawi (Hillocks & Jennings, 2003; Nichols, 1950). This situation changed abruptly in the early 2000s, however, as new outbreaks were reported from mid-altitude (>1000 m above sea level) areas of south-central Uganda (Alicai et al., 2007), western Kenya (H.M. Obiero, personal communication), and northwestern Tanzania (Jeremiah & Legg, 2008), precipitated by massive increases in populations of the whitefly, *Bemisia tabaci* (Legg et al., 2011). CBSD has subsequently been shown to be spreading as a pandemic through the major cassava-growing regions of East and Central Africa (Bigirimana, Barumbanze, Ndayihanzamaso, Shirima, & Legg, 2011; Legg et al., 2011) and threatens to spread further westwards into Central and West Africa (Legg, Somado, et al., 2014).

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2.2.3 Cassava frogskin disease

Root symptoms associated with CFSD have been known in the Amazonas region for many years, but the first serious outbreak was reported in 1971 and occurred in the Cauca region of Colombia, across the Andes, which caused up to 90% yield losses. CFSD is characterized by the failure of the storage roots to accumulate starch and so affected roots develop a rough epidermis resembling the wart-like skin of toads or alligators, hence its name in Spanish (cuero de sapo) and in Portuguese (jacare). Only recently it was found that complex virus infections are associated with root symptoms of CFSD, that several novel viruses are part of that complex, and that neither CsFSaV nor phytoplasma can induce CFSD symptoms or the associated leaf symptoms in single infections (Alvarez et al., 2009; Carvajal-Yepes et al., 2014). More studies are needed to determine the specific role of each component.

As a consequence of this uncertainty, grafting is recommended for CFSD indexing in cassava. Interestingly, CsTLV is a member of the *Torradovirus* genus, is whitefly transmitted and has been associated with leaf symptoms in "Secundina" in single infections (Carvajal-Yepes et al., 2014).

2.3. Vectors of cassava viruses

2.3.1 Cassava mosaic geminiviruses

From the earliest period of research on CMD, it was suspected that the whitefly, *Bemisia tabaci* (Genn.), was the vector of the pathogens causing the disease, and these suspicions were provisionally confirmed by studies in both Central Africa (Kufferath & Ghesquière, 1932) and East Africa (Storey & Nichols, 1938). Dubern (1994) described the characteristics of transmission of ACMV and confirmed that *B. tabaci* was a relatively inefficient vector. Transtadial but not transovarial transmission was demonstrated. Adult whitefly transmission comprised a relatively long (minimum 3 h) acquisition access period (AAP) with a short (minimum 10 min) inoculation access period (IAP). A moderately long latent period (minimum 3 h) was recognized to be a consequence of the circulative transmission pathway.

The persistent mechanism of transmission, typical of begomoviruses, meant that ACMV particles were retained for an experimentally determined time of up to 9 days, a period that in nature might equate to the lifetime of the insect. This long-term association between virus and vector has important consequences for the pattern of spread of CMGs, and notably means that these viruses can be carried over long distances by their whitefly hosts. There have been no specific studies examining long distance flight of

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cassava-colonizing *B. tabaci*, but circumstantial evidence obtained from regional virus spread data suggests that *B. tabaci* populations can carry CMGs over distances of up to 38 km in a year (Legg, 2010). Research on *B. tabaci* elsewhere has reported individual flights of adult whiteflies of up to 7 km (Cohen, Kern, Harpaz, & Ben-Joseph, 1988).

CMGs from south Asia are transmitted in a similar way to their African relatives. ICMV was successfully transmitted from cassava to cassava by whiteflies reared on cassava, but not whiteflies reared on sweet potato (Antony et al., 2006), most likely since these were different *B. tabaci* genotypes. Virus-free cassava, generated by meristem-tip culture, has been used to study the transmission of viruses in cassava by *B. tabaci*. Using cassava-adapted whiteflies, symptoms appeared 25 days after inoculation and 85% of test plants became infected (Duraisamy et al., 2013).

2.3.2 Cassava brown streak viruses

Storey (1939) suggested that *Bemisia* whiteflies were the most likely vector of the viruses causing CBSD, but this was not definitively proved until many years later (Maruthi et al., 2005). Early experiments indicated transmission frequencies of about 22%, but more recent work has achieved higher efficiency levels and has indicated that transmission of these ipomoviruses is semipersistent (Jeremiah, 2012; Jeremiah, C. S. & Maruthi, M. N., unpublished data). Preliminary data from this work indicate a minimum AAP of 5 min, a minimum IAP of 30 min and a maximum retention time of 24 h. No significant differences in transmission have been observed for the two species of CBSVs (CBSV and UCBSV) (M.N. Maruthi, unpublished data). The shorter retention time of CBSVs by *B. tabaci* in comparison with CMGs, suggests that CBSVs are less likely to spread over long distances than CMGs.

2.3.3 Viruses associated with CFSD

There are no published studies of the vector transmission of Latin American viruses. Even the etiology of CFSD has yet to be fully described, as there is currently no confirmation of the vector(s) responsible for transmitting the viruses associated with the disease. Interestingly, it was observed that whiteflies (*Aleurotrachelus socialis* Bondar) were able to transmit agents responsible for leaf symptoms from CFSD-affected plants to healthy "Secundina" plants, but whether root symptoms could be elicited through similar "transmission" remained unclear (Angel, Nolt, & Pineda, 1987, Angel, Pineda, Nolt, & Velasco, 1989).

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2.4. Epidemiology of cassava viruses

2.4.1 Cassava mosaic geminiviruses

During the early years of the Amani program in northeastern Tanzania, it was observed that the rate of spread of CMD was much greater at hot and moist low-altitude locations than it was at higher elevations where temperatures were cooler and the whiteflies less abundant (Storey, 1936, 1939). Similar epidemiological characteristics were noted from Ivory Coast in West Africa, during the intensive period of research of the ORSTOM/CIRAD program (Fauquet & Fargette, 1990; Fauquet, Fargette, & Thouvenel, 1988). Some of the key milestones achieved in developing knowledge of the epidemiology of CMD were as follows:

i. The most important source of new infection in initially CMD-free plantings was shown to be surrounding plantings of cassava (Fargette, Fauquet, Grenier, & Thresh, 1990; Fargette, Fauquet, & Thouvenel, 1985).

ii. Environmental spread gradients were demonstrated in which both whitefly vectors and new CMD infections were aggregated on upwind borders of fields (Fargette et al., 1985, 1990).

iii. Rates of spread were shown to vary greatly between seasons, and most of this variation could be attributed to changes in whitefly abundance and temperature (Fargette, Jeger, Fauquet, & Fishpool, 1993).

iv. A decline in susceptibility of cassava plants to new infection was demonstrated as plants matured (Fargette et al., 1993).

CMGs have been identified from several plants other than cassava (Alabi, Ogbe, et al., 2008; Robertson, 1985). However, the frequency of infection is typically very low, which coupled with the year-round presence of cassava means that cultivated cassava is considered to be the only epidemiologically significant host of CMGs.

As the focus of research switched from West to East Africa during the 1990s, great emphasis was placed on experimenting with CMD-resistant varieties, and large differences in the rate of spread into cassava varieties were demonstrated in Uganda (Otim-Nape, Thresh, & Shaw, 1998). Recovery (disappearance of symptoms during crop growth) and reversion (production of virus-free cuttings by infected parent plants) (Fargette, Thresh, & Otim-Nape, 1994) were shown to be important resistance mechanisms influencing epidemiology.

In Uganda, massive changes in the character of CMD spread were recorded from several fixed locations as an epidemic of unusually severe CMD expanded its geographical range to affect many parts of the country

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(Gibson, Legg, & Otim-Nape, 1996; Legg & Ogwal, 1998; Otim-Nape et al., 1997). Some of the earliest molecular studies of CMGs helped to explain this phenomenon, by demonstrating that this unusually rapid spread of CMD was associated with the occurrence of mixed infections of two CMGs: ACMV and EACMV-UG (Harrison et al., 1997; Zhou et al., 1997). Synergism between the CMGs resulted in raised virus titers and increased virus spread between plants and fields. Arguably the most important "driver" of this rapid virus spread, however, was the massive increase in abundance of the *B. tabaci* whitefly vector that coincided with this outbreak in Uganda (Legg & Ogwal, 1998; Otim-Nape, Thresh, & Fargette, 1996). Molecular evidence based on cytochrome oxidase I sequences of mitochondrial DNA have suggested that genetically distinct populations of *B. tabaci* whiteflies are associated with the CMD pandemic in East and Central Africa (Legg, French, Rogan, Okao-Okuja, & Brown, 2002; Legg, Sseruwagi, et al., 2014).

Early studies of the epidemiology of CMD in India demonstrated primary spread through the use of diseased planting material (Shanta, 1978; Thankappan, 1978) and secondary spread through the *B. tabaci* whitefly vector (Nair, 1985). ICMV was reported to be transmitted by *B. tabaci* from cassava to cucumber (Mathew & Muniyappa, 1993; Menon & Raychaudhuri, 1970) as well as from cassava to cassava (Antony et al., 2006).

2.4.2 Cassava brown streak viruses

It was recognized from the earliest period of CBSD research in the 1930s that the disease spread more readily at low altitudes (Storey, 1936). The rapid spread that has occurred since 2004 in the Great Lakes region has demonstrated the importance of super-abundant populations of the whitefly vector, *B. tabaci* (Legg et al., 2011).

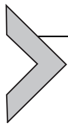
As is the case for the CMGs, the CBSVs appear to have no epidemiologically significant alternative host plants, although *Manihot glaziovii* has been shown to be infected by these viruses (Mbanzibwa et al., 2011). The question of the origins of the CBSVs remains an interesting one from an evolutionary perspective, as cassava arriving in East Africa in the eighteenth century must have been infected from a wild host somewhere in the region.

CBSVs are transmitted semipersistently, which means that they are not carried long distances by vectors, while the persistent transmission of CMGs means that they spread together with migrating populations of *B. tabaci*.

Field trials to determine the field-level epidemiological characteristics of CBSD have shown that gradients of spread are relatively steep from infected source plots to neighboring initially uninfected test plots (Jeremiah, 2012). The highest apparent rates of CBSD spread occurred in the cool, dry part of the year, suggesting that environmental factors (such as temperature, and/or soil moisture) may be important contributing factors to CBSD symptom expression (Jeremiah, 2012).

2.4.3 Latin American viruses

Almost nothing is known about the epidemiology of the viruses affecting cassava in South America (Calvert et al., 2012). It is apparent, however, that most of these viruses are almost entirely propagated through the use of infected planting material. If there were specific and more efficient vectors of these viruses, then they would certainly have become more widespread and spread more readily than they currently do. It is significant, at least for some of the viruses occurring in South America, that the *B. tabaci* genotype groups present in South America ("New World" and "MEAM1"; Dinsdale, Cook, Riginos, Buckley, & De Barro, 2010) do not colonize cassava (Carabali, Bellotti, Montoya-Lerma, & Cuellar, 2005). More importantly, the absence of cassava-colonizing populations of *B. tabaci* in South America means that the likelihood of the CMGs or CBSVs spreading there following inadvertent introduction from Africa is low.



3. MANAGEMENT OF CASSAVA VIRUSES

3.1. Management strategies for plant viruses in cassava

There are several general components that are essential for the effective management of plant virus diseases, and these are equally applicable to cassava. These components can be broadly described as: recognition and monitoring; prevention of infection; and control of infection. An important facet of the context for controlling viruses in cassava is that the majority of farmers growing this crop are subsistence producers who are either unable or choose not to allocate resources to the purchase of inputs for virus control.

3.1.1 Recognition and monitoring

Recognition of the occurrence of a disease is an essential prerequisite for its control. For cassava viruses, the ease with which this can be achieved varies greatly. Some viruses produce very obvious symptoms in the plant's foliage (CMGs, virus combinations producing CFSD), while others are much more

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cryptic, as symptoms may be confined to lower leaves or roots (CBSVs) or may not be visible at all in any plant part (CsSLV). For the major economically important diseases such as CMD in Africa and Asia, and CBSD in Africa, large-scale surveillance programs are required to record incidence levels and their changes over time and geography. These can be achieved effectively for CMD using visual symptoms and standardized survey protocols (Sseruwagi, Sserubombwe, Legg, Ndunguru, & Thresh, 2004), but virus testing may be required to confirm infection status for CBSD.

A vital aspect of recognition and monitoring systems is accurate diagnosis. Effective diagnosis relies on a combination of proper recognition of symptoms—where these are present—and the application of accurate, robust, and affordable laboratory-based diagnostics. Great progress has been made in the development and extension of diagnosis systems for the most important virus groups, such as the CMGs in Africa and Asia, and the CBSVs in Africa. Recent research in Latin America, however, using next-generation sequencing (NGS) approaches, has made it clear that current knowledge of the viruses affecting cassava is incomplete, and further work is required before fully comprehensive virus diagnosis systems can be developed. This will be particularly important for tissue culture-based virus indexing systems where it is necessary to provide assurance that tissue culture material is virus-free, for the purposes of local, regional, or international germplasm exchange.

3.1.2 Prevention of infection

Preventing infection can be an effective method of controlling disease, although in many situations this may be difficult to achieve. Many of the approaches of prevention might be considered as cultural control methods or phytosanitation. This starts with assuring the health of material to be planted, and then by selecting a site not close to other fields that might serve as sources of virus inoculum, or in a location known to be unfavorable for insect vectors. Unlike several other more commercial vegetatively propagated crops, cassava is almost never grown under protected conditions. The only exception to this general rule is when tissue culture plantlets are hardened off, usually following reception from a remote source during germplasm exchange programs. Infection in a newly planted crop can be prevented by assuring that the variety grown is resistant to vector-borne infection, and also by controlling the vectors themselves, although this is rarely practiced for cassava. Resistance may either be to the vector itself or be to infection by viruses carried by the vector. An important feature

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of varieties resistant to CMD is that infection is delayed relative to susceptible varieties. Delaying infection is an important contributing factor to the prevention of virus disease in cassava, notably since yield losses decline the later that cassava plants become infected by virus (Fauquet & Fargette, 1990).

3.1.3 Control of infection

Virus infection in cassava may be controlled by inhibiting virus replication or movement in the infected plant or by destroying infected plants. In the latter approach symptomatic or virus-positive asymptomatic plants are removed, typically through uprooting (=roguing). A wider range of techniques are used to inhibit virus function within infected plants. Meristem tip culture combined with thermotherapy excludes virus particles from meristem tissue, which is subsequently propagated through tissue culture. Both conventional and transgenic resistance approaches rely on modifications of the host plant's inherent defense mechanisms to restrict virus replication and/or movement. While these do not directly eliminate virus particles, they control infection by restricting the multiplication of the virus and thus its concentration to levels that do not result in economic damage or by restricting movement such that only a small proportion of plant parts are affected. Since virus-resistant varieties can be propagated for many years, the deployment of host plant resistance has been the most widely used control tactic in the control of cassava viruses. Although transgenic varieties offer the potential to make this resistance stronger, more efficient and more durable, their widespread use is likely to continue to be constrained by difficulties in resolving regulatory concerns.

In the following sections, we discuss each of the major cassava virus control approaches in greater detail.

3.2. Diagnostics and surveillance

Diagnostic methods used for the detection and identification of the viruses of cassava have been generally similar wherever the viruses occur. During the early years of research, from the 1930s to the 1970s, classical techniques of symptom description, grafting, mechanical inoculation, and the use of indicator plants were used. More recently, serological and nucleic acid-based techniques have facilitated more rapid, sensitive, and high volume diagnostic assessments. As nucleic acid sequencing has become cheaper and more widely accessible, whole genome sequencing has become a common "add-on" to diagnostic testing programs, and "fishing" for asymptomatic virus-like nucleic acids can now be achieved with NGS. This technique

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proved vital in the diagnosis and characterization of viruses associated with CFSD in Colombia (Carvajal-Yepes et al., 2014).

There are several groups of users of cassava virus diagnostics, and each has different requirements, as follows:

i. *Researchers*: diagnostics development; identification of new viruses; virus characterization; detection and identification of viruses in cassava research materials; virus indexing; surveillance; training. *Priorities*: novelty, sensitivity.

ii. *Plant protection/quarantine staff*: testing germplasm imports/exports; local quarantine; testing for seed certification. *Priorities*: accuracy, ease of use.

iii. *Commercial tissue culture laboratories*: virus indexing to assure quality of tissue culture material. *Priorities*: accuracy, ease of use, low cost.

iv. *Extension and NGO agricultural staff*: symptom recognition to provide advice to farmers. *Priorities*: ease of use in the field, robustness.

v. *Farmers*: symptom recognition to help in field management of cassava virus diseases. *Priorities*: ease of use in the field, robustness, and simplicity.

Although there has been great progress in the development and application of diagnostics for cassava viruses that can be used in laboratory situations by researchers and plant protection officers, there are currently no field-based testing options that can be used at "point-of-use" by extensionists or farmers themselves.

3.2.1 Cassava virus diagnostics in Africa

A number of diagnostic procedures utilizing symptoms, electron microscopy, serological methods and polymerase chain reaction (PCR)-based methods have been used for the diagnosis of CMD and CBSD (Alabi et al., 2011; Deng, McGrath, Robinson, & Harrison, 1994). Reliability, sensitivity, and operational convenience have led to the emergence of PCR or reverse transcription (RT)-PCR-based methods as the mainstay for routine diagnosis of CMGs and CBSVs, respectively (Abarshi et al., 2012, 2010; Alabi, Kumar, & Naidu, 2008; Aloyce, Tairo, Sseruwagi, Rey, & Ndunguru, 2013; Mbanzibwa et al., 2011; Monger, Seal, Cotton, & Foster, 2001; Were, Winter, & Maiss, 2004; Zhou et al., 1997). High-sequence diversity (intraspecific homology of ca. 85–99%; and ca. $<75\%$ nucleotide sequence homology between species) was such that early diagnostic methods for CBSVs encountered challenges such as "false negatives" due to mismatches in primer alignment. The enrichment of the public database with an increasing number of sequences for CBSV and UCBSV

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obtained from different locations in seven countries has enabled the development of oligonucleotide primers against the most conserved motifs, thereby improving the robustness of PCR-based diagnostic assays for CBSVs (e.g., Abarshi et al., 2012). The greatest challenge for the diagnosis of the CMGs is the relatively large number of species (9 in Africa), which means that current multiplex PCR technologies are unable to resolve all species in a single test. However, a multiplex PCR assay developed for the simultaneous detection of ACMV and EACMV-like viruses occurring in Africa (Alabi, Kumar, et al., 2008) was found to be useful for virus indexing due to its broad specificity. Another multiplex PCR method described can detect up to four CMG species in a single reaction (Aloyce et al., 2013), and protocols have also been described for the simultaneous detection of the two species of CBSVs and the two most widely distributed species of CMGs—ACMV and EACMV (Abarshi et al., 2012).

Real-time RT-PCR techniques have opened up the potential for increased throughput and the more sensitive detection of UCBSV and CBSV. Real-time Taqman assays developed for this purpose have been extensively used for the detection of CBSVs in cassava planting material produced for distribution within the countries of the Great Lakes region (Adams et al., 2013). Reverse transcription loop-mediated isothermal amplification (RT-LAMP)-based methods have also been developed for the detection of CBSVs (Tomlinson et al., 2013) and have the important practical advantage that they do not require thermal cycling equipment. These methods have been further modified through incorporating labeled primers for rapid detection of amplification products using lateral flow devices containing antibodies specific to the incorporated labels, avoiding the need for fluorescence detection or gel electrophoresis.

3.2.2 Cassava virus diagnostics in South Asia

A number of diagnostic methods utilizing symptoms, serology, and nucleic-acid-based techniques have been employed for the detection of CMGs in India. Several attempts have been made to detect ICMV using serological techniques (ELISA, DIBA, and TBIA) (Makeshkumar & Nair, 2001, Malathi et al., 1985; Malathi, Varma, & Nambisan, 1989), and histochemical and fluorochrome staining of infected tissues have also been used (Govindankutty, 2004). Different formats of PCR and multiplex PCR have been applied for the detection of ICMV and SLCMV (Anitha et al., 2011; Dutt, Briddon, & Dasgupta, 2005; Hegde, Jeeva, Makeshkumar, Misra, & Veena, 2010; Makeshkumar, Anoopsankar, Nair, & Edison, 2005). Nucleic

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acid spot hybridization techniques have been employed to detect CMGs in India for screening large numbers of samples (Makeshkumar et al., 2005). Most recently, real-time PCR methods have been developed and used for the detection of low titers of SLCMV in cassava plants showing symptom recovery, as well as for virus quantification (Deepthi, Makeshkumar, Unnikrishnan, & Winter, 2012).

3.2.3 Cassava virus diagnostics in Latin America

Viruses in Latin America for which sequence information is available are now being routinely detected by RT-PCR. Except for CsTLV isolates, the technique has proved to be efficient in detecting single infections and has significantly shortened the time for virus indexing when compared to the graft-indexing protocol using the cassava landrace indicator "Secundina" (Carvajal-Yepes et al., 2014). The great variability found among CsTLV isolates in Colombia alone means that different primer combinations are required to detect both RNA1 and/or RNA2 (Carvajal-Yepes et al., 2014; Verbeek et al., 2012). More sequences of diverse isolates need to be characterized in order to improve the effectiveness and reliability of CsTLV diagnostics.

For some cassava viruses reported in the 1980s (CsSLV, CsCaMV, CsCSLV, and CsALV), there are no sequences or antisera available (Table 1) and thus identification by indexing is impractical since it would require time-consuming and expensive virus purification and electron microscopy, and in some cases would not be possible at all. Moreover, some of these viruses could be related to (and possibly even the same as) the novel viruses recently identified. It is therefore suggested that they should be removed from the list of quarantine viruses that is used when controlling movements of cassava germplasm.

3.2.4 Cassava virus surveillance

Surveillance is an activity that has most commonly been implemented by research teams, and most frequently in Africa as a means to track the presence and national or regional spread of the CMGs and CBSVs. Some of the earliest countrywide surveys were implemented in Uganda at the time of the outbreak of the severe CMD associated with mixed ACMV+EACMV-UG infections (Otim-Nape et al., 1998). Similar approaches were also used in South Asia to determine the patterns of distribution and spread of ICMV and SLCMV (Patil et al., 2005). As the scale of the CMD pandemic in Africa became apparent, surveillance systems were increasingly organized at the

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regional (multicountry) level, within the framework of large CMD mitigation programs. This process led to an increasing standardization of protocols used which enabled datasets to be compared between countries. The first program to take this approach, focusing primarily on CMD, was a USAID-funded initiative that operated in eight countries of East and Central Africa between 1998 and 2008 (Legg, Kapinga, Teri, & Whyte, 1999; Legg, Owor, Sseruwagi, & Ndunguru, 2006). The most recent regional surveillance initiatives have combined assessments of both CMD and CBSD in countries of East, Central, and Southern Africa. Results from these were used to generate an online map database (IITA, 2012). Concerns about the speed with which results are made available, together with a demand for increased involvement of agricultural workers and farmers at the level of the communities affected, led to the piloting of alternative surveillance strategies. One example, referred to as the "Digital Early Warning Network," was employed for early warning of CBSD in northwestern Tanzania and Rwanda and combined farmer training with the use of a phone-based SMS reporting system (IITA, 2011a).

3.3. Quarantine systems

The frequency and volumes of cassava germplasm exchanged between and within counties are increasing dramatically, due to the increasing demands from crop diversification and crop improvement programs, and also to address insufficiency in planting material in food insecure parts of sub-Saharan Africa. This activity, however, involves an inevitable risk of accidentally introducing viruses along with the host plant material. Risk of pest and pathogen spread along with cassava germplasm is very high because:

- **i.** The crop is clonally propagated.
- **ii.** Cassava germplasm is predominantly exchanged as stem cuttings both within and even between countries, especially in sub-Saharan Africa.
- **iii.** There is a high dependence on inter- and intracontinental exchange of cassava germplasm for research, crop diversification, and cassava improvement.
- **iv.** A number of virus species associated with the cassava diseases in Africa, Asia, and Latin America are geographically restricted to those continents therefore representing threats to others (Table 1).
- **v.** Vectors (e.g., *B. tabaci*) occur widely and are capable of spreading viruses from accidentally introduced sources.
- **vi.** Knowledge of the distribution and diversity of several cassava viruses is limited and reliable diagnostics are not available (Table 1).

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vii. Capacities of plant protection/quarantine organizations, as well as extension systems and the growers themselves, are currently insufficient for adequate monitoring of germplasm for pests and pathogens, and for the implementation of emergency control measures in case of accidental introductions.

Recent cases of the introduction of EACMV-like viruses into Indian Ocean islands (De Bruyn et al., 2012) and Oman (Khan, Akhtar, Al-Matrushi, Fauquet, & Briddon, 2013) are examples of virus spread that can be directly attributable to humans carrying infected planting material. Additionally, the emergence of the CBSD pandemic in the Great Lakes region of East/Central Africa demonstrates how the effects of natural spread by whiteflies can be significantly augmented through the propagation of infected planting material (Legg et al., 2011).

Technical guidelines for the safe movement of cassava germplasm have been established to minimize the risk of virus spread during germplasm exchange activities (Table 2) (Frison, 1994; Frison & Feliu, 1991). This requires the active engagement, however, of both national and regional plant protection bodies, working through established regulatory frameworks on germplasm exchange.

The measures listed in Table 2 have the potential to minimize the risk of spread of well-characterized viruses and also poorly characterized viruses,

Table 2 Basic guidelines for safe exchange of cassava germplasm

- The transfer of germplasm should be carefully planned in consultation with quarantine authorities and should be in amounts that allow adequate handling and examination. The material should be accompanied with the necessary documentation.
- Under no circumstances should germplasm be moved as rooted plant material except for *in vitro* plantlets.
- Cassava germplasm can be moved as seed, pathogen-tested *in vitro* material, or as cuttings from reestablished pathogen-tested *in vitro* material that has been grown under containment.
- All germplasm should be collected from healthy-looking plants and when possible from areas where quarantine pests are not present. Source plants should be tested for endemic viruses (e.g., CMGs and CBSVs for material sourced in East and Southern Africa)
- Only under special circumstances should the movement of untested, vegetative material be considered.
- Germplasm from areas where viruses of quarantine concern are known to occur should go through intermediate or postentry quarantine.

Source: Frison and Feliu (1991).

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and even the exchange of planting material of unknown health status. In the absence of virus-free sources, planting material should be generated using thermotherapy and meristem culture techniques, which have been shown to eliminate viruses such as CMGs and CBSVs (IITA, 2010b; Kartha & Gamborg, 1975; Mohammed, Abarshi, Hillocks, & Maruthi, 2012; Zapata, Miller, & Smith, 1995) and regenerate virus-free cassava plants for international exchange. Recently, generic methods based on the high-throughput sequencing of viral RNAs have been established to overcome this bottleneck and discover viruses without any prior knowledge of their occurrence in host plants (Kreuze et al., 2009).

At local level, there have been emergency situations where it has been essential to speed the movement of virus-resistant germplasm from one country to neighbors. Such a situation occurred during the early stages of regional spread of the CMD pandemic from Uganda to neighboring countries. In this situation, open quarantine sites were established at locations in Tanzania and Kenya that were close to their respective borders with Uganda (Mohamed, 2002). These carefully managed sites received large quantities of CMD-resistant germplasm that was at the time only available in Uganda and provided a cheap and effective means of exchanging germplasm and safely multiplying resistant varieties in the target countries. Aspects of the exchange that were critical to its success were the fact that CMD symptoms can be readily seen in visual assessments thereby greatly minimizing the likelihood of inadvertent virus introductions, and that the pandemic-associated virus, EACMV-UG, had already spread into these border areas prior to the introduction of the new germplasm. Similar approaches have been proposed for the exchange of CBSD-resistant varieties between neighboring countries in East Africa. These have so far not been approved, however, in view of the more cryptic symptoms of CBSD and the greater likelihood, therefore, of inadvertently introducing CBSVs together with the exchanged germplasm.

3.4. Phytosanitation and clean seed

Phytosanitation typically refers to the set of measures that may be used to ensure the health of crop plants. With respect to cassava, there are three major components, which will be examined in turn.

3.4.1 Producing virus-free planting materials

3.4.1.1 Meristem tip culture and thermotherapy

Several methods have been used to produce virus-free planting material. High temperatures are known to inhibit replication in many virus species.

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This phenomenon is exploited in cassava by growing young plants at a constant temperature of 35 °C for 1 month, and then excising 0.3-mm meristem tips and growing them on in tissue culture (Frison, 1994). These or similar procedures, coupled with virus indexing, are now used widely by laboratories working with tissue culture cassava where there is a need to ensure that the viruses are excluded from tissue culture material.

3.4.1.2 Field application of thermotherapy and hot water treatment

There have been various efforts to use this same principle to clean up cassava cuttings in field settings. Heat treatment and hot water treatment have been shown to eliminate certain CMGs in some cassava varieties (Gibson, 1994; Kaiser & Louie, 1982), but the "recovery" rates have been incomplete (i.e., a proportion remain infected after being returned to the normal temperature) and the approach has been shown to be ineffective in eliminating severe infections resulting from the cooccurrence of more than one CMG species. There has consequently been no widespread application of this technique for cassava virus control.

3.4.1.3 Field propagation of virus-free stocks of "clean seed"

Stocks of planting material (also referred to as "seed") may be "cleaned-up" in the field through regular monitoring and removal of infected plants. The effectiveness and speed of this "clean-up" process depends on several factors, such as the initial virus incidence in starting material, the abundance of whitefly vectors, the resistance of the variety to virus infection, and the distance of the field from neighboring (and potentially infected) cassava fields (Bock, 1994c; Thresh, Otim-Nape, & Fargette, 1998). By manipulating these factors, a conducive environment for the production of "clean seed" can be established, and within the space of two to three growing seasons, it can be possible to establish a "clean seed site" that is virtually virus-free. This approach is currently being implemented at a pilot level in Tanzania, for the production of "prebasic" "seed" of elite cassava varieties, and in the light of promising preliminary results, is being extended to other countries that have a similar goal of raising the quality standards of cassava planting material production.

3.4.2 Managing the health of cassava crops in the field

The relative merits of applying tactics for the management of the health of cassava crops in the field will vary depending on the nature of the viruses that affect those crops. CMD and CBSD provide a notable example of this.

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Phytosanitation measures may be easy to apply for CMD, since clear foliar symptoms mean that it is straightforward to identify infected plants, but the benefits of phytosanitation may be limited, since crops may be readily infected by whiteflies arriving from distant inoculum sources. Conversely, phytosanitation measures may be less readily applied for CBSD, since symptoms are cryptic, but the benefits may be great, since cutting-borne infection is relatively more important than infection carried in by vectors from remote inoculum sources. This indicates that a thorough understanding of the epidemiology of cassava viruses is a key prerequisite to determining the most appropriate management strategy.

3.4.2.1 Roguing

Roguing is the removal of unwanted plants from within a planted crop. Plants are typically removed either because they are the wrong variety or because they are diseased. Roguing can be used to reduce levels of virus infection in cassava crops where the infecting virus(es) express clear foliar symptoms. The procedure is unpopular with farmers, and therefore hard to promote. Despite the fact that roguing is widely recommended by researchers and extension services trying to encourage cassava virus control, there is little evidence to suggest that it has real benefit for an individual farmer applying the tactic independently of his or her neighbors. Roguing in farmers' fields may provide little yield advantage for resistant varieties, but for susceptible varieties, it may have a significantly negative impact, because of the greatly reduced plant population that results from high virus incidence levels (Mallowa, Isutsa, Kamau, & Legg, 2011). The situation may be different; however, where the primary "product" of the field is planting material rather than tuberous root yield, since in such a situation the value of obtaining "healthy" planting material with little or no virus infection may outweigh the cost of the reduced plant population.

3.4.2.2 Selection

Selection usually refers to the choice by a grower or an agricultural worker of asymptomatic vegetative propagules—mature stems in the case of cassava. Like roguing, this practice was recommended for many years (Calvert & Thresh, 2002), although in contrast to roguing, field data have been presented to indicate that selection of healthy planting material results in significantly increased yields, even in relatively small plots (Mallowa et al., 2011). Studies on CMD in both Ivory Coast and Kenya indicated that the outcome of initiating cassava plantings with healthy cuttings was dependent on the

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inoculum pressure environment into which the crop was planted (Bock, 1994b; Fauquet, Fargette & Thouvenel, 1988). In India, virus-free stocks have been obtained by rigorous selection and through the use of meristem-tip therapy. These have been used in experiments and shown to remain largely free of CMD in areas where there is limited spread by whiteflies and substantial increases in yield have been achieved in this way (Nair, 1990; Nair & Thankappan, 1990). In Latin America, where the viruses affecting cassava are generally less aggressive and less readily spread by vectors, selection of healthy planting material has been widely applied for virus disease management (Calvert & Thresh, 2002). Vigorous selection has been shown by researchers to be effective in controlling CFSD, but many farmers are not inclined to inspect tuberous roots (where CFSD symptoms are expressed) before they collect and distribute the stem cuttings.

3.4.2.3 Crop management and disposition

Several crop management strategies can help to minimize infection of cassava crops in the field. Most act through reducing the likelihood of vector-borne infection. Where there are options to plant at different times of the year, dates can be selected which are least favorable for vector spread. For CBSVs, the relatively steep spread gradients mean that isolation of a new crop from neighboring fields can significantly reduce the degree of spread into the new crop (Jeremiah, 2012). Although the benefit of isolation appears to be less for CMGs, experimental trials have nevertheless demonstrated a strong reduction in infection within newly planted crops with increasing distance and reduced frequency of surrounding inoculum sources (Legg et al., 1997). In view of the obvious benefits of isolation, this has become an important requirement for the establishment of "clean seed" sites of cassava in recent years.

3.4.2.4 Intercropping

Intercropping is widely practiced by cassava growers in Africa, but is less frequent in the more commercially oriented production systems of Latin America and Asia. Several experimental studies have investigated the potential beneficial effect of intercropping on cassava virus control in Africa. Although some have suggested that there are significant benefits in intercropping either maize or cowpeas with cassava (Fondong, Thresh, & Zok, 2002), the benefits appear to be unpredictable and vary depending on the pattern of planting and mixture (Fargette & Fauquet, 1988). Perhaps

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in view of these mixed results, intercropping has not generally been advocated for the control of cassava viruses.

3.4.3 Implementing large-scale phytosanitation initiatives

Several organized and geographically extensive phytosanitation schemes have been implemented for the management of cassava viruses. These fall into two categories—certification and eradication.

3.4.3.1 Certification

Certification schemes are used for a wide range of crops as a means of assuring the quality of "seed" products. Interest in developing the commercial potential of cassava, combined with a recognition of the severe deleterious effects caused by viruses on planting material, have encouraged the pilot-scale development of programs promoting quality of cassava "seed." A set of guidelines designed to help growers produce cassava to defined quality standards, including virus disease tolerance levels, was first developed in the late 1990s in Uganda. This "Quality Management Protocol" (QMP) was first widely used within the framework of a regional cassava and banana disease mitigation project, the "Crop Crisis Control Project," which was implemented from 2006 to 2007 in six countries of East and Central Africa. The use of the QMP approach was extended during the follow-on Great Lakes Cassava Initiative (2007–2011) (IITA, 2011b), during which the system was broadened to include routine testing of CBSVs at higher level "seed" propagation sites. The application of this quality management system was effective in minimizing levels of virus disease at all stages of "seed" multiplication.

3.4.3.2 Eradication

All of the economically important cassava viruses (CMD, CBSD, and CFSD) are widely distributed and affect thousands, if not millions of cassava farms. In addition, some have significant reservoirs in noncassava plant hosts. Complete eradication of any of these is therefore an unrealistic goal for the foreseeable future. However, attempts have been made historically to achieve at least the regional-level eradication of some of these viruses. The best example of this is the large-scale replacement of heavily CMD-affected local cassava varieties by improved CMD-resistant material in northeastern Uganda in the 1950s. The removal of local materials was enforced by colonial authorities, with severe penalties imposed for failure to comply. Although the mode of implementation was authoritarian, the

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program greatly reduced the impact of CMD in that region of Uganda in subsequent years, and was considered to be a success.

It is clearly evident that the benefits of reducing virus disease inoculum levels accrue as the spatial scale of the application of these measures increases. Moreover, the benefits to be gained from area-wide phytosanitation for the control of CBSD are potentially greater than they would be for CMD, since the semipersistent transmission of the CBSVs means that they are less readily spread over long distances than CMGs. Based on this theoretical background, a pilot scheme to test community-wide phytosanitation was initiated in Tanzania in 2012. A key element of this ongoing program will be comprehensive community sensitization and collective planning, to ensure that all cassava growers in the four villages targeted in the first phase are aware of the stages of the process. The ambitious goal of the disease control work in each village will be to completely replace existing stocks of virus-diseased cassava plants with newly introduced virus-free planting material of the best virus-resistant varieties available. Since quantities of new material will initially be limiting, dissemination of the new material (coupled with removal of existing diseased material) will be achieved in a rolling process, in which new material initially received by the "Primary Recipient Group," will be shared in a second season with a larger "Secondary Recipient Group," who will in turn share planting material with any remaining cassava farmers in the village. If the approach proves to be successful, it may offer great potential for scaling up, and ultimately for regionwide reductions in the impact of cassava virus diseases.

3.5. Conventional breeding for resistance

3.5.1 Introduction

Efforts to improve cassava yield are generally not geared toward the highest possible yield under favorable conditions, but rather toward obtaining stable yields in marginal conditions where cassava is grown at present and is likely to expand in future. To achieve this, breeders address the key constraints of cassava production. Among the virus diseases, CMD, CBSD, and CFSD are currently the major constraints reducing cassava production, and these have been the target of resistance breeding work since the time that they were first recognized. Early successes that were achieved in this work provided encouragement for its further development, and this component of cassava virus disease management has consequently received most research attention and investment. Although vectors are a key component in the pathosystems of each of the major cassava virus groups, relatively less effort has been

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directed toward identifying sources of vector resistance and incorporating them into cassava germplasm.

3.5.2 Breeding for resistance to CMD in Africa

Breeding for CMD resistance was initiated in Tanzania (at Amani, near Tanga) by Storey in 1937 (Nichols, 1947) and was sustained through to 1958. The resistance of cultivated cassava to CMD was low in that part of Tanzania, as it was elsewhere in Africa. This encouraged researchers to exploit resistance sources from wild cassava relatives through interspecific hybridization. Moderately resistant clones with reasonable yield were selected from among the progeny of third backcrosses to cassava of ceara rubber (*M. glaziovii*) by cultivated cassava hybrids. Higher CMD resistance was subsequently obtained by intercrossing among resistant selections probably because it concentrated recessive genes (Jennings & Iglesias, 2002). These clones were consequently referred to as the "Amani hybrids."

Seeds of clone 5318/34 bred by the Amani group were introduced into Nigeria in 1956, and clone 58308 was subsequently selected as resistant to CMD in 1958 (Beck, 1960; Ekandem, 1970). Though breeding was discontinued in 1961, 58308 was maintained and it continued to show CMD resistance for nearly 30 years (Hahn & Theberge, 1985). However, this clone yielded poorly and produced low-quality tuberous roots (Hahn, Howland, & Terry, 1973).

The International Institute of Tropical of Agriculture (IITA)'s Roots and Tubers Improvement Program, established in 1971, gave priority to cassava, and its breeding strategy was to incorporate disease resistance into susceptible but well-adapted local cultivars. More crosses between clone 58308, local cultivars, and *M. glaziovii* were made. The improved families from these crosses showed superior resistance to CMD. After 10 years of research, the goal of developing high-yielding CMD-resistant material that increased yields of small-scale farmers by up to 40% was achieved (Nweke, 2009). Varieties TMS 30395, TMS 63397, TMS 30555, TMS 4(2)1425, and TMS 30572 were released in 1977 (Nweke, 2009), and TMS 30359 and TMS 30001 were reported to be almost immune to CMD (Hahn & Theberge, 1985).

During the 1990s, breeding work was extended to many of the major cassava-producing countries of Africa, through partnerships between IITA and national research programs. CMD gene pyramiding generated varieties with increasingly high levels of resistance, and many of these were selected for local environmental suitability in target countries and made available to farmers (Dixon et al., 2008, 2010; Ntawuruhunga et al., 2006).

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The inheritance of CMD resistance was initially not well understood due to cassava's genetic heterogeneity and the limited amount of research dedicated to this topic. *M. glaziovii*-derived resistance was initially reported to be multigenic or polygenic (Doughty, 1958; Hahn, Howland, & Terry, 1977). Hahn et al. (1977) subsequently indicated that the resistance appeared to be additive with heritability of about 60% and that it was recessive (Hahn, Terry, & Leuschner, 1980). This polygenic, recessive resistance derived from *M. glaziovii* was subsequently designated CMD1, while a second source obtained from local Nigerian landraces was shown to be conferred by a major dominant gene and named CMD2 (Akano, Dixon, Mba, Barrera, & Fregene, 2002). Breeding lines expressing CMD1 are not immune to infection by CMGs but express mild or transient symptoms as a result of incomplete systemic infection leading to reversion of symptoms (Fargette et al., 1994). Several of these genotypes are moderately susceptible to CMD, especially when infected by more than one CMG species (Thresh & Cooter, 2005). West African landraces from which the CMD2 resistance source was derived (TME lines) show high levels of resistance to CMD, but the single gene character of this source of resistance means that it is more vulnerable to resistance breakdown than CMD1. By combining CMD1 and CMD2, breeders have been able to produce varieties with the agronomic and organoleptic qualities desired by cassava growers, while also possessing very high levels of durable CMD resistance (Dixon et al., 2003; Rabbi et al., 2014).

3.5.3 Breeding for resistance to CBSD in Africa

Breeding for resistance to CBSD was initiated together with the work on CMD during the Amani program in Tanzania (Nichols, 1947). A similar approach was used, in which resistance genes from wild relatives were introgressed and then backcrossed into cultivated cassava (Jennings, 2003; Nichols, 1947). One of the most resistant products of this program was the variety 46106/27 (Jennings, 1994; Nichols, 1947). Its resistance to CBSD has persisted up to the present in farmers' fields in coastal East Africa, where it is known locally as "*Kaleso*" in Kenya and "*Namikonga*" in Tanzania (Hillocks & Jennings, 2003).

CBSD resistance was recently reported to have both additive and nonadditive genetic effects. However, the additive effects were more important, implying that intrapopulation selection methods should be effective in accumulating favorable alleles in breeding materials (Moreno-Gonzalez & Cubero, 1993). In three different studies, Kaleso showed the highest general combining ability for resistance to CBSD (Kulembeka

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et al., 2012; Mtunda, 2009; Munga, 2008). This cultivar is now widely used by national breeding programs in the region to generate CBSD-resistant genotypes.

Following the outbreak of CBSD in the Great Lakes region in 2004, screening for CBSD resistance was initiated in Uganda using a large set of CMD-resistant families (Ntawuruhunga, Kiryowa, Okechukwu, Otim-Okello, & Kanju, 2012). CMD-resistant genotypes (MM and MH series) gave varied responses to CBSD, based on their foliar and root symptoms, and three categories of resistant (reduced incidence of symptom expression), tolerant (reduced severity of symptom expression) and susceptible genotypes were identified. Historically, much of the breeding work to combat CBSD has focused on tolerance, since resistance to infection is rare even in introgressed interspecific hybrids, and the expression of foliar symptoms has been considered as acceptable if root symptoms are absent, infrequent, or very mild. Yield loss experiments have indicated that the reduction in growth resulting from foliar symptoms can lead to larger reductions in yield than spoilage of roots due to CBSD-associated root damage (Hillocks, Raya, Mtunda, & Kiozia, 2001). This suggests that future breeding work needs to place greater emphasis on identifying sources of resistance to infection, which will prevent expression of symptoms in both foliage and roots. Recent studies have suggested an overlap in the resistance status of "tolerant" and "resistant" varieties, however, as concentrations of CBSVs in CBSD-tolerant varieties, expressing only mild foliar symptoms, have been shown to be significantly lower than concentrations of CBSVs in susceptible varieties (Maruthi, Bouvaine, Tufan, Mohammed, & Hillocks, 2014). This suggests that "tolerant" varieties possess molecular resistance mechanisms that impair the replication of CBSVs.

Several CBSD-resistant clones have been identified in Kenya (*Kaleso*, *Guzo*, *Gushe*, *Kibiriti Mweusi*, and *Ambari*), Mozambique (*Nikwaha*, *Chigoma Mafia*, *Nachinyaya*, *Xino Nn'goe*, *Likonde*, *Mulaleia*, and *Badge*), and Tanzania (*Namikonga*, *Kiroba*, *Nachinyaya*, *Kigoma Mafia*, *Kitumbua*, *Kalulu*, *Mfaransa*, *Muzege*, *Gezaulole*, and *Kibangameno*). These are now being used extensively in the respective countries' breeding programs as sources of resistance to generate new improved clones. Intercrossing among them concentrates resistance genes and allows recessive genes to be expressed (Hillocks & Jennings, 2003). Some of the derived resistant F1s have the ability to remain free of CBSD symptoms when exposed to infection and when infected the incidence and severity is low. Such F1s have been officially released in Kenya, Mozambique, Tanzania, and Uganda. Due to the current low levels

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of resistance available from *M. esculenta*, future efforts will also focus on evaluating and tapping natural resistance from wild relatives of cassava.

3.5.4 Breeding for resistance to CMD in South Asia

In India, cassava breeding is mainly carried out at the Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, as well as in the State Agricultural Universities through the All India Coordinating Centers (Abraham, Nair, & Naskar, 2000; Unnikrishnan, EaswariAmma, Sreekumari, Sheela, & Mohan, 2002). During the last five decades of research at CTCRI, a large number of cassava varieties with varying reactions to ICMV and SLCMV have been released (Abraham et al., 2006; George, Kumar, & Unnikrishnan, 2012; Nair et al., 1998).

Mnga-1 is a breeding line from IITA (TMS 30001), which was received via CIAT in 1994 and which has been continuously evaluated for its response to Indian CMD under natural field conditions, showing high levels of resistance (Unnikrishnan et al., 2002). It was released for cultivation in Tamil Nadu as *Sree Padmanabha* and has also been used for developing more resistant clones through intervarietal hybridization. The evaluation of seedling populations of Mnga-1 for ICMV and SLCMV resistance resulted in 242 CMD-resistant lines with high yield and starch content. Two of these lines—CMR1 and CMR129—have proved to be popular among farmers (Unnikrishnan et al., 2011).

Fifty-six clones with resistance derived from West African landraces (CMD2) were introduced to India from the International Center for Tropical Agriculture (CIAT, Colombia) in 2005. These clones were crossed with inbred lines with the aim of developing heterotic hybrids. The CMD-resistant hybrids (CR21-10, CR43-11, and others) are now being evaluated in different parts of India under the All India Co-ordinated Research Project—Tuber Crops (Sheela, Abraham, & Moorthy, 2012).

In an interspecific breeding program, *M. glaziovii*, *Manihot caerulescens*, *Manihot tristis*, *Manihot flabellifolia*, *Manihot peruviana*, and *Manihot pseudoglaziovii* were used for developing improved CMD resistance. Among them, hybrids of *M. caerulescens* exhibited the highest levels of resistance and were used as donor parents for transferring resistance to elite Indian cultivars (Sheela, EaswariAmma, Unnikrishnan, & Nair, 2002; Sheela, Unnikrishnan, Edison, & EaswariAmma, 2004; Unnikrishnan et al., 2002). Among these crosses, one interspecific hybrid cassava with *M. caerulescens* (CMC-1)

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showed a very high level of resistance to CMD. Prebreeding lines developed at CTCRI also offer future potential for developing new CMD-resistant high-yielding hybrids.

3.5.5 Breeding for resistance to CFSD in Latin America

Field studies carried out by CIAT, Colombia show that there is a gradient of resistance to CFSD among cassava landraces. It also seems that different degrees of severity can be observed even between plants of a single cassava variety, which is almost certainly a consequence of the complex nature of the disease, comprising several types of mixed infections. In a 10-year assay carried out at CIAT's experimental station at Santander de Quilichao, Cauca, Colombia, more than 400 varieties were screened for resistance to CFSD. After a period of 5 years of evaluation, 70% of the landraces showed resistance to CFSD, including genotypes from Brazil, Peru, Colombia, and Paraguay (M. Cuervo and L. Calvert, unpublished data). In a further 3-year period of evaluation, these "resistant" landraces only showed very mild symptoms or no symptoms at all in the roots. To ensure high levels of disease pressure during this study, plants were inoculated by grafting using cuttings of a plant (code CM5460-10) showing severe CFSD root symptoms. When analyzing a sample of this inoculum using recently developed molecular techniques, mixed infections of at least four different viruses were identified, in addition to CsFSaV (Carvajal-Yepes et al., 2014). These preliminary results provide a strong indication that it is possible to control CFSD by the identification and use of varietal resistance. Molecular breeding through the identification of markers associated with resistance/susceptibility and better characterization of the causal agent(s) of the symptoms observed in CFSD-affected plants would improve the management of the disease.

3.5.6 The deployment of virus-resistant cassava varieties in Africa

Following the successes achieved in the development of CMD-resistant varieties in the 1970s, IITA worked with national program partners to distribute and evaluate these materials in many of the major cassava-producing countries of Africa (Manyong, Dixon, Makinde, Bokanga, & Whyte, 2000). Much of the country-based work involved various levels of selection, starting either from seed or clonal stages. Ultimately, on-farm trials with farmers completed evaluation procedures, and the best performing and most farmer-preferred varieties were formally released. As the capacities of national programs throughout Africa have grown stronger, an increasing

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level of independence of country-level breeding programs has been achieved, as crossing blocks have been established, and novel breeding strategies designed and implemented. As a consequence, the breeding process has become more sensitive to local environmental conditions, including differences in the presence of virus species and strains.

Between 1970 and 1998, it was estimated that 208 cassava varieties were released, many of which were selected primarily on the basis of resistance to CMD (Manyong et al., 2000). Effective partnerships were critical to the successful implementation of this geographically extensive program of germplasm exchange and evaluation (Ntawuruhunga et al., 2013; Nweke, 2009). Key partners included: national research systems, government and NGO extension agencies, subregional research organizations, and regional root crops networks such as the East Africa Root Crops Research Network (EARRNET) and the Southern Africa Root Crops Research Network (SARRNET).

After 1990, much of the large-scale variety deployment work in Africa was targeted toward the mitigation of virus disease pandemics, starting with the severe CMD pandemic in the early 1990s, and subsequently shifting emphasis to the CBSD pandemic from 2006 onward. Some of the most significant programs that were established for this purpose are summarized in Table 3. It is significant that over the period that these initiatives have been active, there has been a gradual increase in the scale of support and in the diversity of activities undertaken. There has been a significant impact in terms of the level of adoption of CMD-resistant varieties. Adoption was greatest in Uganda (where the National Agricultural Research Organization [NARO] first initiated large-scale dissemination of CMD-resistant varieties in 1993), where more than 50% of surveyed cassava farmers were growing CMD-resistant varieties by 2007 (Omongo et al., 2007). Similarly, while the frequency of CMD-resistant varieties grown as the predominant variety in eastern DR Congo was only 4.9% in 2007 (Tata-Hangy et al., 2007), by 2011 it was greater than 50% in six of the seven districts assessed (IITA, 2012). These changes have had a significant impact on the overall incidence of CMD in this part of East and Central Africa. Consequently, while average CMD incidence in Uganda was 83% in 1999 (Sseruwagi et al., 2004), by 2011 it had fallen to 12.9% (IITA, 2012).

Unfortunately, as farmers began to experience the yield benefits of effective CMD management provided by resistant varieties, the new outbreak of CBSD began to spread through the Great Lakes region. Since 2006, regional cassava virus disease management programs have therefore focused

Table 3 Regional cassava virus disease mitigation programs in sub-Saharan Africa: 1998–2017

Short title	Main themes	Executing institution[a]	Partner institutions[a]	Target countries[a]	Duration	Donor	Approx. budget ($)
Emergency mitigation of the CMD pandemic	Surveillance; varietal selection; germplasm exchange and multiplication; open quarantine; awareness raising	IITA	EARRNET, NARS, NPPOs, UA, CBOs, Extension	Bu, Ke, Rc, Rw, Tz, Ug	1998–2008	USAID	4,000,000 (400,000 per year)
Crop Crisis Control Project (C3P)	Surveillance; participatory varietal selection; germplasm multiplication; awareness raising; quality management	CRS	IITA, EARRNET, NARS, CBOs, Extension	Bu, Dc, Ke, Rw, Tz, Ug	2006–2007 (18 mths)	USAID	4,500,000 (Cassava part: 1,500,000 per year)
Great Lakes Cassava Initiative (GLCI)	Surveillance; participatory varietal selection; germplasm multiplication; awareness raising; quality management; CBSD focus; seed systems; virus diagnostics	CRS	IITA, NARS, NRI, FERA, KEPHIS, GTIL, CBOs	Bu, Dc, Ke, Rw, Tz, Ug	2007–2012	BMGF	22,000,000 (5,500,000 per year)
New Cassava Varieties and Clean Seed (5CP)	Germplasm exchange, germplasm evaluation; clean seed systems	IITA	NARS, NRI, KEPHIS, TOSCI, GTIL	Mw, Mz, Ke, Tz, Ug	2012–2016	BMGF	5,700,000 (1,425,000 per year)
Biotechnology applications to combat CBSD	QTL Mapping of CBSD resistance; RNASeq; identification of CBSD resistance genes; multilocation evaluation of resistance; marker-assisted breeding	IITA	ARI-Tanzania; NaCRRI	Ug, Tz	2009–2016	BMGF	3,900,000 (557,000 per year)
Building capacity in cassava virus diagnostics	CMD and CBSD virus diagnostics; physical and human capacity strengthening; surveillance; epidemiology; sustainable virus management	MARI	NARS, NCSU, NRI, RU	Ke, Mw, Mz, Rw, Tz, Ug, Zm	2009–2017	BMGF	11,000,000 (1,375,000 per year)

[a]Bu, Burundi; Dc, Democratic Republic of Congo; Ke, Kenya; Mw, Malawi; Mz, Mozambique; Rw, Rwanda; Tz, Tanzania; Ug, Uganda.
IITA, International Institute of Tropical Agriculture; CRS, Catholic Relief Services; MARI, Mikocheni Agricultural Research Institute.
EARRNET, East Africa Root Crops Research Network; NARS, National Agricultural Research Systems; NPPOs, National Plant Protection Organizations; UA, University of Arizona; CBOs, Community-based Organizations; NRI, Natural Resources Institute; FERA, Food and Environment Research Agency; KEPHIS, Kenya Plant Health Inspectorate Service; TOSCI, Tanzania Official Seed Certification Institute; GTIL, Genetic Technologies International Limited; ARI-Tanzania, Agricultural Research Institutes of Tanzania; NaCRRI, National Crop Resources Research Institute; NCSU, North Carolina State University; RU, Rutgers University.

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increasingly on this. Since very little was known about the molecular character, biology and epidemiology of the viruses causing CBSD, considerable emphasis was placed on investing in critical research to strengthen knowledge about CBSD. The absence of good sources of resistance coupled with the greater potential for the application of phytosanitation have both favored an emphasis on developing seed systems that produce high-quality virus-free planting material. The longer term goal, however, will be to develop varieties that combine high levels of resistance to both CMD and CBSD. Conventional, gene editing, and transgenic approaches to breeding are all being explored with this goal in mind.

3.6. Molecular breeding using next-generation methods

Recent advances in NGS technologies have driven down sequencing costs and increased sequence capacity at an unprecedented rate (Varshney, Nayak, May, & Jackson, 2010). By harnessing these new technologies, molecular breeding can predict phenotypes from genotypes more efficiently and with greater accuracy than before. Some molecular breeding techniques rely on *a priori* knowledge of molecular markers associated with a trait (such as marker-assisted selection), and others, such as genomic selection (Goddard & Hayes, 2007; Heffner et al., 2009; Lorenz et al., 2011; Meuwissen, Hayes, & Goddard, 2001), use all markers across the genome to predict the performance of individuals (Jannink, Lorenz, & Iwata, 2010; Meuwissen et al., 2001). Genomic selection is being tested in cassava (Ly et al., 2013) with particular weighting for virus resistance in Uganda and Nigeria.

Efforts have been made to associate traits with markers, and early studies identified a microsatellite (SSRY28) and an RLFP locus (GY1) that flanked the single dominant CMD resistance gene known as CMD2 (Akano et al., 2002). These, together with additional markers (Fregene et al., 2006), were used to introgress CMD2 into Latin American germplasm for deployment in Africa (Okogbenin et al., 2007). Recently, Rabbi et al. (2014) using a high-density genotyping approach identified SNP locus S5214_780931 as being closest to the QTL peak for CMD2 and showed that all previous resistance linked markers cooccurred in the same chromosomal location indicating a single source of monogenic resistance. Other quantitative recessive resistance has been introgressed using purely conventional methods from wild *M. glaziovii* (Jennings, 1957, 1994; Nichols, 1947), although QTLs have not currently been identified for this source of resistance.

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Efforts are underway to identify molecular markers associated with resistance and tolerance to CBSD in varieties including *Namikonga* (*Kaleso*), *Nachinyaya*, and *Kiroba* using a bi-parental QTL mapping approach. This approach is being supplemented by sequencing of the transcriptomes (RNASeq) of CBSV and UCBSV infected and control resistant and susceptible genotypes, with the aim of identifying candidate genes putatively involved in the resistance mechanism. Further functional genomics approaches are being implemented to validate these candidate genes.

Recent efforts are emphasizing the development of varieties with triple resistance to CMD, CBSD, and the whitefly vector. Accurate and robust phenotyping of germplasm for the multiple viral agents involved in CMD and CBSD is one of the greatest challenges. However, phenotyping procedures have been recently strengthened through the use of the *Agro*-inoculation of CMGs (Bi, Aileni, & Zhang, 2010) and a grafting method for CBSD (Wagaba et al., 2013), coupled with real-time PCR assays for quantification of CBSVs (Adams et al., 2013) and CMGs (Otti, Owati, Melaku, & Kumar, 2013). These novel techniques are greatly helping to improve our understanding of varietal response to virus and speeding the process of germplasm selection.

It is envisaged that as genomics tools are applied and the molecular basis of resistance/tolerance to virus diseases becomes better understood, greater progress will be made in deploying different combinations of resistance sources to enhance effectiveness and durability.

3.7. Transgenic approaches to strengthening host plant resistance

3.7.1 Introduction

The development of virus-resistant farmer-preferred cultivars is restricted by limitations inherent to traditional breeding (Ceballos, Iglesias, Perez, & Dixon, 2004). Transgenic technologies offer an alternative and novel way for introgressing beneficial traits into cassava. The concept of "pathogen-derived resistance" proposes that transforming plants with a pathogen's gene will disrupt the "normal" host–pathogen relationship and generate resistance against the pathogen (Sanford & Johnson, 1985). Powell-Abel et al. (1986) first demonstrated pathogen-derived resistance in transgenic tobacco plants conferring resistance against tobacco mosaic virus (TMV).

3.7.2 Transgenic approaches to developing CMD resistance

Moderate levels of resistance against several geminivirus species have been demonstrated in transgenic tobacco and tomato expressing the AC1 gene,

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which encodes the replication-associated protein (Rep) (Asad et al., 2003; Brunetti et al., 1997; Hong & Stanley, 1996; Noris et al., 1996; Sangare, Deng, Fauquet, & Beachy, 1999). Chellappan, Masona, Vanitharani, Taylor, and Fauquet (2004) reported that transgenic cassava expressing the ACMV AC1 gene showed resistance to both homologous and heterologous species of cassava-infecting CMGs. The high levels of resistance against CMGs were correlated with posttranscriptional gene silencing (PTGS) through the production of transgene-specific siRNAs. Zhang, Vanderschuren, Fütterer, and Gruissem (2005) demonstrated that transgenic cassava expressing antisense RNAs of ACMV Rep (AC1), TrAP (AC2), and REn (AC3) could resist ACMV infection via PTGS.

Vanderschuren et al. (2007) and Vanderschuren, Alder, Zhang, and Gruissem (2009) reported that the siRNAs, homologous to either the common region or AC1 in transgenic cassava, were able to suppress the replication of ACMV, leading to recovery after infection or immunity to infection by the virus. As the common region of ACMV does not share a high degree of sequence homology with other CMGs (e.g., EACMV and SACMV), the resistance was expected to be strain specific (Vanderschuren et al., 2007). In the last two decades, RNAi-based approaches have been tested in transgenic cassava and proved to confer robust CMD resistance in the model cassava cultivar TMS 60444. Constructs developed in this way need to be transferred to farmer-preferred cultivars, although the stability of the engineered CMD resistance remains to be demonstrated under field conditions and over multiple cycles of propagation.

3.7.3 Transgenic approaches to developing CBSD resistance

Several research groups have demonstrated that CBSD resistance can be engineered in cassava using RNAi-based approaches targeting the CP sequence of CBSVs (Ogwok et al., 2012; Vanderschuren, Moreno, Anjanappa, Zainuddin, & Gruissem, 2012; Yadav et al., 2011). Yadav et al. (2011) demonstrated that at least one of the two ipomoviruses, UCBSV, can be efficiently controlled using RNAi technology. Transgenic cassava plants constitutively expressing siRNA targeting the near full-length coat protein (FL-CP) of UCBSV showed 100% resistance to UCBSV in glasshouse experiments (Yadav et al., 2011) and confined field trials in Uganda (Ogwok et al., 2012). The transgenic plants expressing siRNA targeting the near FL-CP showed a 3-month delay in disease development, with 98% of clonal replicates remaining symptom free over the 11-month trial, whereas all nontransgenic control plants developed CBSD symptoms on aerial tissues by 6 months after planting. Highly effective suppression

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of UCBSV in transgenic plants under field conditions suggested that the coexpression of siRNAs from the CP sequences of both UCBSV and CBSV within the same plant holds promise for the integration of robust field resistance to CBSD into farmer-preferred cassava cultivars (Ogwok et al., 2012). The Donald Danforth Plant Science Center and IITA, in partnership with NARS in Uganda and Kenya, are developing and testing CBSD-resistant cassava using local farmer-preferred varieties under the Virus Resistant Cassava for Africa project (Taylor et al., 2012). In the most recent dataset, at least three transgenic lines showing near immunity to CBSV and UCBSV have been identified (Odipio et al., 2014).

Recently, Vanderschuren et al. (2012) demonstrated that sequences of the CP are highly conserved between CBSV and UCBSV and can therefore be used to engineer resistance against both viral species in the cassava model cultivar TMS 60444. This technology was transferred to the Nigerian cassava landrace TME7, which has natural resistance to CMD. This combination of natural and engineered virus resistance can be a promising approach to combat multiple cassava viral diseases in Africa (Vanderschuren et al., 2012). The RNAi approach is therefore a promising technology for engineering resistance to CBSD in farmer-preferred landraces. However, its value is greatly augmented where RNAi-derived transgenic resistance to CBSD is combined with conventionally bred CMD resistance. Many African countries have yet to finalize regulatory guidelines for the introduction, testing, and field planting of genetically modified (GM) crop plants, but the generally positive view that many African governments take of the practical benefits offered by GM mean that significant progress in resolving regulatory constraints is likely to be achieved in the near future.

3.8. Vector control

All of the economically important cassava viruses in Africa and South Asia are transmitted by the whitefly vector, *B. tabaci*. In spite of this fact, relatively little research attention has been directed toward developing strategies to control this insect. Host plant resistance, cultural methods, biological control, insecticides, and combinations of these tactics in integrated strategies are all used to counter the physical and virus-vectoring damage caused by whiteflies on other crop plants that are affected by whitefly transmitted viruses, such as cotton (Ellsworth & Martinez-Carrillo, 2001) or tomatoes (Lapidot & Friedmann, 2002). Expensive control methods, such as the application of insecticides, are almost never practiced by farmers in Africa since

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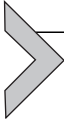
the cassava crop is primarily grown for subsistence purposes, rather than as a commercial crop. Some attention has also been given in India to the use of insecticides to control the whitefly vector in attempts to reduce the spread of CMD. However, the results have been unsatisfactory, and the routine use of insecticides is considered to be inappropriate on health and environmental grounds and for these reasons is not currently recommended (Malathi et al., 1985; Thankappan, Makeshkumar, & Edison, 1997).



4. CONCLUSIONS

Cassava has been grown for approximately 10,000 years, but it was only after its introduction to Africa in the sixteenth century, followed by Asia two centuries later, that the plant took on the role of a key component in global food systems. In the current environment of global warming—as one of the principal features of anthropogenic climate change—cassava is likely to become increasingly important (Jarvis, Ramirez-Villegas, Campo, & Navarro-Racines, 2012). Set against this, pests and diseases have become a major factor limiting production. For the pests, the greatest problems have arisen from inadvertent movements to places in which they did not previously occur. Outbreaks and epidemics of diseases have also arisen from external introductions (cassava bacterial blight), but more importantly from the movement of local viruses (CMGs and CBSVs) into the cassava crop. A key determining factor behind these scenarios is the adaptation of whitefly vectors to cassava, which has so far only happened in Africa and South Asia. The recent pandemics of severe CMD and CBSD in Africa seem to be associated with a second stage of adaptation of *B. tabaci* whiteflies to cassava, which has resulted in 100-fold increases in vector numbers in many areas and continues to affect new areas each year. Climate change threatens to exacerbate this situation still further, although studies are required to determine what the overall effects are likely to be on plant, vector, and viruses.

Set against this worrying future scenario is the rapid progress that has been made in both understanding the viruses of cassava and developing and delivering control solutions. This progress has been particularly significant in the last two decades, partly as a consequence of the increased severity of cassava virus problems. Some of the key requirements for future efforts to tackle cassava viruses are as follows:

i. *Awareness*. Highlight the continued importance of cassava viruses, their potential to recombine producing novel more virulent strains, and the

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risks posed by their inadvertent spread from country to country and continent to continent. Set up early warning networks to respond to transcontinental introductions and encourage preemptive measures to mitigate the westward spread of CBSD in Africa.

ii. *New technology*. Maximize the use of novel techniques both for virus/vector characterization, as well as for the development of resistant varieties through conventional, gene editing, and transgenic approaches. Combine resistance genes in order to ensure durability.

iii. *Surveillance*. Develop cheaper and more robust diagnostics to facilitate targeted but more frequent surveillance programs for monitoring new outbreaks and tracking progress in controlling them.

iv. "*Seed systems*." Invest in strengthening the quality and geographical coverage of "seed" systems for cassava to bring about widespread reductions in inoculum levels.

v. *Community action*. Work with communities in areas affected mainly by CBSD and CFSD to aim for local eradication through the implementation of area-wide phytosanitation programs.

vi. *Whitefly control*. Prioritize research on the integrated management of *B. tabaci* whitefly vectors—an area of work that has been neglected in recent decades.

vii. *Capacity development*. Train a cadre of researchers, plant protection officers, and agricultural development workers in order to equip them to drive the research and development activities necessary for the successful long-term management of cassava viruses. Training is required in all cassava-growing regions, but the needs are particularly acute in Africa.

Cassava is a highly adaptable crop that will continue to serve humanity into the future as a key source of food and industrial products. Viruses have posed a major challenge to this role. Research successes achieved over the last two decades, however, coupled with plans for future work such as those highlighted above, give us good reason to be hopeful about the future prospects, not just for virus management, but for the overall production of this important crop.

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