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Cassava Mosaic and Brown Streak Diseases: Current Perspectives and Beyond

Chrissie Rey[1] and Hervé Vanderschuren[2]

[1]School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg 2000, South Africa; email: chrissie.rey@wits.ac.za

[2]AgroBioChem Department, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium; email: herve.vanderschuren@ulg.ac.be

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cassava, CMD, CBSD, disease resistance, virus, genetic engineering

Abstract

Cassava is the fourth largest source of calories in the world but is subject to economically important yield losses due to viral diseases, including cassava brown streak disease and cassava mosaic disease. Cassava mosaic disease occurs in sub-Saharan Africa and the Asian subcontinent and is associated with nine begomovirus species, whereas cassava brown streak disease has to date been reported only in sub-Saharan Africa and is caused by two distinct ipomovirus species. We present an overview of key milestones and their significance in the understanding and characterization of these two major diseases as well as their associated viruses and whitefly vector. New biotechnologies offer a wide range of opportunities to reduce virus-associated yield losses in cassava for farmers and can additionally enable the exploitation of this valuable crop for industrial purposes. This review explores established and new technologies for genetic manipulation to achieve desired traits such as virus resistance.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) originates from South America (1); it was introduced from Brazil into West Africa by Portuguese sailors in the sixteenth century (2) and expanded into East Africa, India, Indonesia, and the Philippines in the eighteenth century. Cassava is mainly grown in tropical regions of South America, sub-Saharan Africa, India, and Southeast Asia (**Figure 1**). It is the most important source of food in developing countries after maize, rice, and wheat and is mainly grown by subsistence and small-scale farmers. A perennial root crop, cassava provides food for an estimated 800 million people (3). Over the period from 1980 to 2013, global cassava production increased from 124 to an estimated 263 million tons (4). Asia, Africa, and the Americas contribute 33.5% (88.2 million tons), 54.8% (144.2 million tons), and 11.6% (30.5 million tons) of world production, respectively (4). Cassava is well adapted to poor soils and is drought resistant. The ability to store roots for lengthy periods in the ground allows flexible harvesting over long periods, making cassava a major food security crop in developing countries. In addition, cassava is the second most important source of starch in the world and has potential industrial uses, such as in animal feed, biofuel, paper, textile, and food processing applications; it can contribute to economic transformation in developing countries where cultivation occurs (5).

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the main biotic and economically important constraints on cassava cultivation in sub-Saharan Africa (6, 7). CMD is widespread in sub-Saharan Africa and the Indian subcontinent (reviewed in 7, 8), whereas CBSD has been reported from East African countries and around the Great Lakes region (**Figure 1**). Leaf

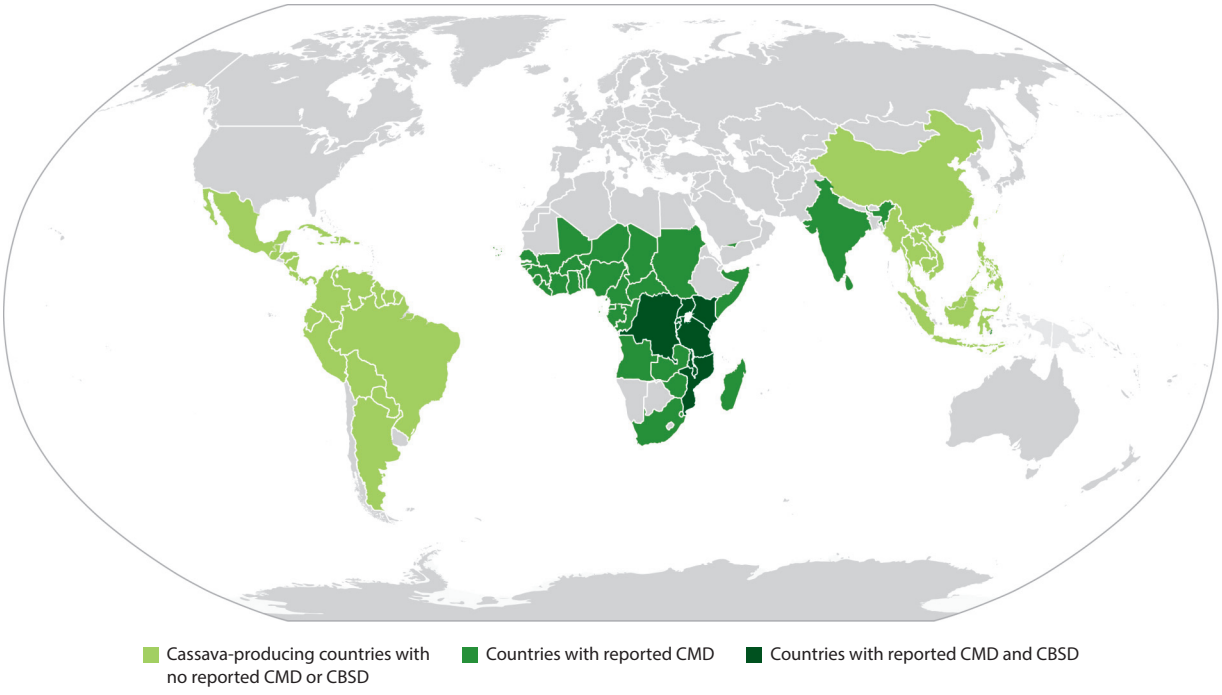

Figure 1

Global distribution of major cassava cultivation areas, and occurrence of cassava mosaic disease (CMD) and cassava brown streak disease (CBSD).

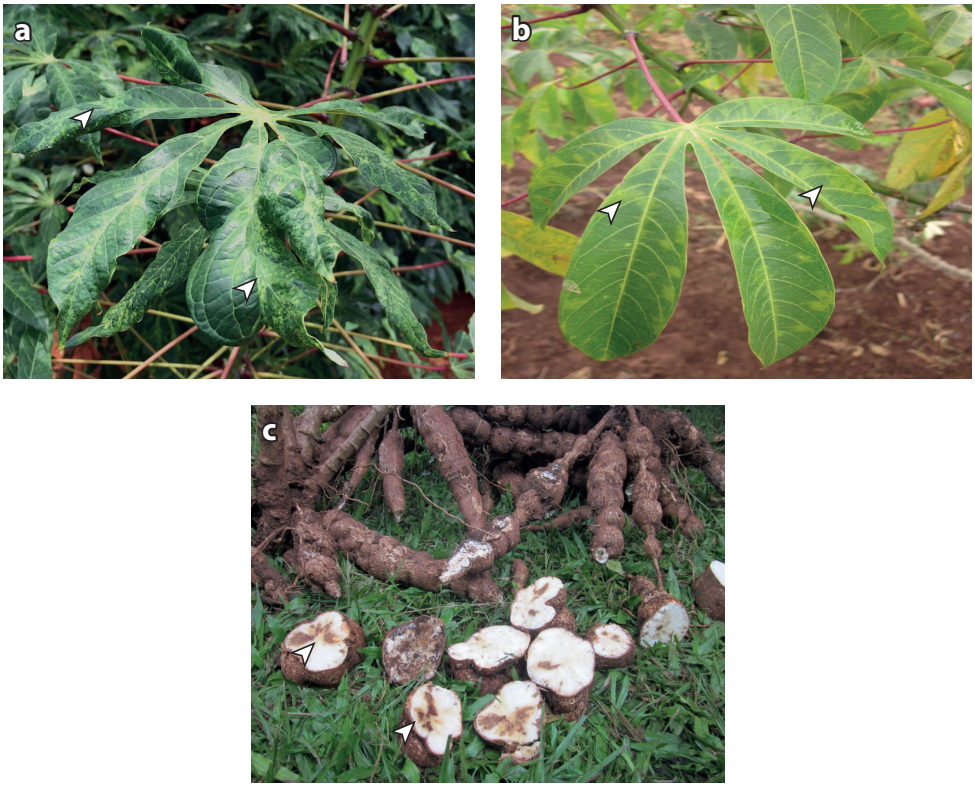

Figure 2

(*a*) Leaf curl, distortion, and yellowing symptoms (*arrowheads*) of cassava mosaic disease in cassava. (*b*,*c*) Symptom types in cassava brown streak disease include (*b*) yellowing (*arrowheads*) of older leaves along the major veins and (*c*) brown, corky, necrotic lesions (*arrowheads*) within the storage roots.

symptoms of CMD in cassava vary from mild mosaic to more severe symptoms of leaf curl, leaf distortion, yellowing, and plant stunting (**Figure 2*a***), depending on the infected cassava cultivar, the virus genotype(s) (mixed infections are common), and the climatic conditions. The major symptom types in CBSD include yellowing of older leaves along the major veins (**Figure 2*b***); round or elongated brown streak-like lesions on the stems; and, most destructively, production of brown, corky, necrotic lesions within the storage roots (**Figure 2*c***) (9). Root and leaf symptoms of CBSD are highly variable depending on host genotype, virus species, and environmental conditions (10, 11). Coinfection with more than one virus species or strain is a common feature in the etiology of CMD, often leading to an increase in symptom severity. Reversion or recovery in cassava is attributed to incomplete systemic movement of the causal viruses, such that whole branches or individual shoots can be symptomless and free of any detectable virus and can provide uninfected cuttings (12).

Both diseases are transmitted by the polyphagous cryptic whitefly complex *Bemisia tabaci* (Gennadius) (*Hemiptera*: *Aleyrodidae*) (13, 14). The cassava mosaic geminiviruses comprise several circular ssDNA viral species belonging to the genus *Begomovirus*, family *Geminiviridae*, and are not seed borne (15). Both diseases are also mechanically transmitted through grafting and sap inoculation of herbaceous plant species (10, 16, 17). In addition, both CMD and CBSD are propagated through infected stem cuttings. A key feature of the geographical areas severely affected by CMD and CBSD is the presence of large whitefly populations on cassava plants. The persistent

mechanism of transmission of cassava mosaic geminiviruses by *B. tabaci* (viruses can be retained up to 9 days) facilitates long-distance movement of virus populations (up to 38 km in a year) (18) and has important consequences for the pattern of spread of cassava mosaic geminiviruses.

KEY DISCOVERIES AND MILESTONES IN THE ELUCIDATION AND CHARACTERIZATION OF CASSAVA VIRAL DISEASES

The increased risk posed by cassava mosaic and brown streak viruses in Africa and Asia due to wide geographical spread and genetic diversification is currently viewed as one of the greatest global threats to cassava growers. Because a wealth of documented literature exists on CMD and CBSD (reviewed in 7, 8), this review highlights what we consider to be the significant milestones over the past decade leading to the current state of knowledge about these two major diseases and their associated viruses. Strategies for new approaches to disease management are also presented.

Cassava Mosaic Disease

CMD is widely considered to be the most significant disease causing yield loss in cassava; *African cassava mosaic virus* was included on the list of the top 10 plant virus pathogens published by *Molecular Plant Pathology* in 2011 (19). In this section, we present some landmark discoveries, from the first report of CMD in the late nineteenth century to the role of recombination and mixed infections in the evolution of multiple cassava-infecting begomovirus species and strains that exist today.

First field study reports. The first report in 1894 of CMD in Africa can be traced to Tanzania (20), and later studies were carried out by Storey & Nichols (15). In West Africa, CMD was first recorded in the coastal areas of Nigeria, Sierra Leone, and Ghana in 1929 and had spread northward by 1945 (21). By the end of the 1940s, CMD had been reported in most of the cassava-growing countries in sub-Saharan Africa (22). Symptoms were described based on the severity of the disease they elicited, and the whitefly *B. tabaci* was identified as the probable transmitting vector (15).

CMD in India has a more recent history; the first report was in 1956 (23). Compared with losses in Africa, losses associated with CMD in India have been relatively moderate, between 10% and 15%, and the disease is restricted to southern India (Kerala and Tamil Naidu and to a lesser extent Karnataka and Andhra Pradesh) (24). Etiology and symptoms of CMD in Asia are similar to those reported in Africa.

The causal agent: discovery of cassava latent virus. A huge milestone in the elucidation of CMD was the first identification of a causal agent, in Kenya, originally suspected to be a virus named cassava latent virus (25). Early efforts to transmit the agent failed, but later, Bock & Woods (26) were able to prove Koch's postulates, and geminivirus-like particles (viewed by electron microscopy) were isolated and mechanically transmitted. They induced mosaic symptoms in *Nicotiana benthamiana*, and the virus was renamed *African cassava mosaic virus*. The first sequence of African cassava mosaic virus (ACMV)-Kenya was subsequently determined (27). The sequencing of ACMV-Kenya was significant and led to more than two decades of intensive research into the structure and function of cassava mosaic geminivirus genes and encoded proteins (28–30).

It is now known that CMD is caused by several circular ssDNA cassava mosaic geminivirus species belonging to the genus *Begomovirus* in the family *Geminiviridae*. The bipartite genome of cassava mosaic geminiviruses is encapsidated in 30×20 nm twinned icosahedral particles ($T = 1$) (**Figure 3*a***) and consists of two covalently closed, circular ssDNA molecules (DNA-A

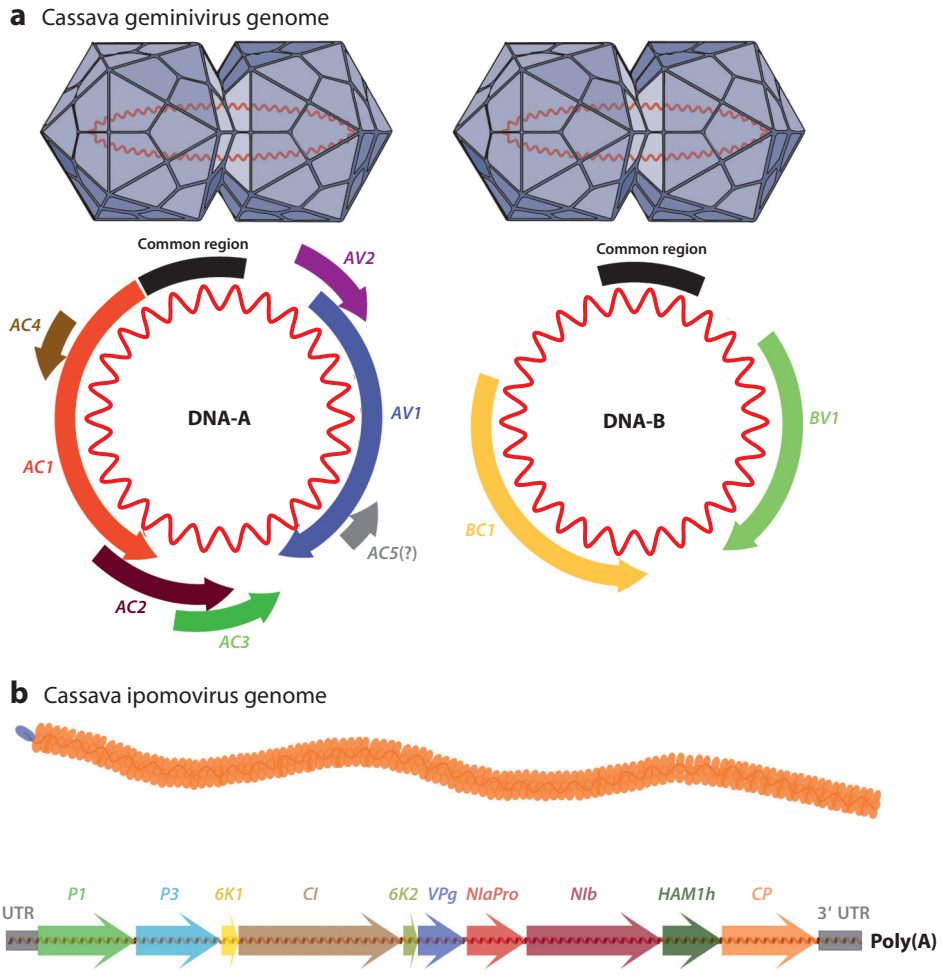

Figure 3

(*a*) Genome organization and structure of cassava geminivirus twinned incomplete icosahedral particles ($T = 1$). DNA-A: *AV2* [encodes putative protein kinase (PKC)], *AV1* (encodes coat protein), common region, *AC1* [encodes replication initiation protein (Rep)], *AC2* [encodes transcription activator protein (TrAP)], *AC3* [encodes replication enhancer protein (REn)], *AC4* (encodes silencing suppressor protein), *AC5* (may encode silencing suppressor protein). DNA-B: *BV1* [encodes nuclear shuttle protein (NSP)], common region, *BC1* [encodes movement protein (MP)]. (*b*) Genome organization and structure of cassava ipomovirus flexuous particles: 5′ untranslated region (UTR), *P1* (encodes serine proteinase protein), *P3* (encodes third protein, unknown function), *6K1* (encodes 6-kDa protein, unknown function), *CI* (encodes cylindrical inclusion protein), *6K2* (encodes 6-kDa protein, unknown function), *VPg* (encodes viral genome-linked protein), *NIaPro* (encodes nuclear inclusion proteinase), *NIb* (encodes nuclear inclusion polymerase), *HAM1h* (encodes reduction of mutation rate protein), *CP* (encodes coat protein), 3′ UTR.

and DNA-B) of approximately equal size (~2.7–2.8 kb) (**Figure 3*a***). Cassava mosaic geminivirus DNA-A has two genes (*AC1* and *AC2*) on its virion-sense strand and four genes (*AC1–4*) on the complementary-sense strand. Rep (encoded by *AC1*) is a critical multifunctional protein involved in initiation of rolling circle amplification. Approximately 350 isolates from 28 distinct begomovirus species were recently annotated to contain a fifth putative open reading frame

(ORF), *AC5*, that is located downstream of *AC3* on the complementary strand of DNA-A, overlapping the region encoding the coat protein (31). Although a role in pathogenicity (as a suppressor of RNA silencing) has been suggested for the AC5 protein from *Mungbean yellow mosaic India virus* (31), the function of AC5 in cassava mosaic geminiviruses is unknown and warrants further investigation. The DNA-B component is required for inter- and intracellular movement and carries two genes; *BV1* encodes a nuclear shuttle protein, and BC1 encodes proteins required for cell-to-cell movement of the virus. The nonanucleotide TAATATTAC sequence where rolling circle replication is initiated is found in the common region of DNA-A and DNA-B and is conserved among members of the family *Geminiviridae* (32). Additional details regarding the structure and function of encoded proteins are reviewed in Reference 28.

From one to eleven cassava mosaic virus species. The role of epidemiology in the emergence of many cassava mosaic geminivirus species, strains, and variants (33) is widely acknowledged. Genome-wide nucleotide pairwise identities of 91% and 94% are the demarcation threshold for cassava mosaic geminiviruses belonging to different species and strains, respectively (33). Since the identification of *African cassava mosaic virus* in sub-Saharan Africa (27), six more species have been revealed in the cassava-growing countries of Africa and the southwest Indian Ocean islands (reviewed in 34). These are *East African cassava mosaic virus* (35), *East African cassava mosaic Kenya virus* (35), *East African cassava mosaic Malawi virus* (36), *East African cassava mosaic Zanzibar virus* (35), *Cassava mosaic Madagascar virus* (37), and *South African cassava mosaic virus* (38). In addition, several other important isolates have been identified, notably the *African cassava mosaic virus/East African cassava mosaic virus* recombinant variant East African cassava mosaic virus (EACMV)-Uganda (39); EACMV-Cameroon [Cameroon-1998] (40); and, more recently, ACMV–Burkina Faso (41). Notably, the cassava viruses in the northeastern part of the Democratic Republic of Congo (Yangambi province) are also able to infect two leguminous species (42), which has implications for further genetic diversification and disease etiology.

Two species occur on the Asian subcontinent: *Indian cassava mosaic virus*, isolated from central and southern India (24), and *Sri Lankan cassava mosaic virus*, initially reported from Sri Lanka (43, 44). Despite the emergence of new strains or variants, no new cassava mosaic geminivirus species in cassava have been reported since the identification of those two species. Though *Sri Lankan cassava mosaic virus* was first discovered in Sri Lanka, it later was found in southern India (45), likely due to movement of contaminated material. Recently, *Sri Lankan cassava mosaic virus* was also reported in Cambodia, the first report of a cassava mosaic geminivirus in Southeast Asia (46). *Jatropha curcas* is a small woody non–food oil seed crop plant belonging to the *Euphorbiaceae* family and suffers from *Jatropha curcas* mosaic disease (JcMD), a newly emerging disease that has been reported in Africa (Nigeria) (47) and India (48). The disease incidence is particularly significant on the Indian subcontinent: approximately 25% in northern India and up to 47% in southern India. A strain of *Indian cassava mosaic virus*, Indian cassava mosaic virus (ICMV)-Dharwad, is the causative pathogen of JcMD in southern India. Recently, another highly pathogenic *Indian cassava mosaic virus* strain, ICMV-Singapore, was identified as the causative agent for JcMD in Southeast Asia; this strain shares 94.5% identity with ICMV-Dharwad (49). The recurrent identification of ICMV as an epidemic viral pathogen in various *Jatropha* plantations warrants further attention, as this virus could potentially recombine with geminiviruses from the natural host.

Origin of cassava mosaic geminiviruses and the role of recombination and mixed infections in diversity. Whereas high genetic diversity (more than 56 variants or strains) has been reported for *East African cassava mosaic virus*, due at least in part to the fact that variants of this species have most frequently been the recombination recipients of DNA-A and DNA-B fragments from

other cassava mosaic geminivirus species, sub-Saharan *African cassava mosaic virus* variants have displayed substantially less diversity, suggesting that East Africa may be the center of cassava mosaic geminivirus diversity (50). *African cassava mosaic virus* most likely evolved in West Africa earlier than *East African cassava mosaic virus* given that cassava was introduced earlier in this region (sixteenth century) than in East Africa (eighteenth century) (2).

The emergence of cassava mosaic geminiviruses throughout cassava-growing regions in sub-Saharan Africa likely results from the introduction of cassava into regions with infected non-indigenous crop species and wild plants harboring indigenous begomoviruses. *B. tabaci* vectors, found on cassava and other indigenous hosts (51), could have transmitted begomoviruses from natural hosts to cassava. Movement of infected vegetative cassava material between geographic regions would have exacerbated mixed infections, creating abundant opportunities for the evolution of synergistic interactions between the coinfecting viruses and for frequent recombination and genome component reassortment to occur. Recombination among cassava mosaic geminiviruses on the African continent and southwest Indian Ocean islands has been well documented (reviewed in 8, 52). Recently, further evidence of recombination between bipartite and monopartite begomoviruses, giving rise to ACMV–Burkina Faso, was reported (41). Although the important contribution of recombination to geminivirus evolution is well established, it remains elusive why recombination contributes to the emergence of cassava mosaic geminivirus complexes and how recombinants with enhanced pathogenicity establish themselves. Several factors, including (*a*) the propensity of begomoviruses to recombine, (*b*) the frequent introduction of polyphagous whitefly types into novel regions, and (*c*) the coadaptation of whitefly types to new cultivated crops and indigenous host plants (53–55), could contribute to the emergence of new recombinant virulent cassava mosaic geminiviruses in the future.

Defective interfering DNAs. The discovery of defective interfering (DI) DNAs associated with DNA-B of ACMV-Kenya in *N. benthamiana* was the first report of a DI DNA modulating infectivity of a bipartite begomovirus (56). Although DIs contain the intergenic region found in all geminiviruses, they are dependent on a helper virus for replication, movement, and transmission. A second DI DNA-B has been associated with *South African cassava mosaic virus* in naturally infected field cassava plants in South Africa, with *BV1* (nucleotide positions 57–1429) entirely deleted and *BC1* partially deleted at its C terminus, leaving only 82% of the gene intact (34). DIs derived from DNA-A are exclusively associated with monopartite geminiviruses, with the exception of the DNA-A-derived DI of EACMV-[TZ15] (57). The demonstration that *African cassava mosaic virus* DI DNAs (approximately half the size of full-length DNA-B) interfere with replication of both genomic components in *N. benthamiana* (58) was a significant finding. Despite this discovery more than 25 years ago, exploitation for control of CMD has not materialized due to the limitations of cognate helper virus specificity.

Sequences that enhance cassava mosaic disease symptoms. The discovery of two novel episomal DNA sequences that enhance CMD symptoms, designated SEGS1 and SEGS2 (for sequences enhancing geminivirus symptoms), has opened up even more potential obstacles in developing and maintaining CMD resistance. SEGS1 breaks resistance in a cassava landrace carrying the *CMD2* tolerance locus when it is coinoculated with EACMV-Uganda (59). Both sequences have no homology to geminiviruses and enhance CMD symptoms when coinoculated with ACMV, EACMV-Cameroon [Cameroon-1998], or EACMV-Uganda. Episomal forms of both sequences were detected in cassava mosaic geminivirus–infected cassava, but not in healthy cassava. SEGS2 episomes were also found in virions and whiteflies (59). The cassava genome contains a sequence that is 99% identical to full-length SEGS1 and three sequences that are 84–89% identical to

SEGS2 and together encompass all of SEGS2 except for a 52-bp region, which includes the episomal junction and a 26-bp sequence related to α satellite replication origins (59). Besides these four sequences, multiple copies of varying lengths of endogenous SEGS1 and SEGS2 are widely distributed in the sequenced cassava genome and are in close proximity or overlapping with cassava genes, suggesting a possible role in regulation of specific biological processes (60). The sequence features of endogenous SEGS are unique but resemble nonautonomous transposable elements such as miniature inverted-repeat transposable elements and helitrons. Furthermore, many SEGS-associated genes, some involved in virus-host interactions, are differentially expressed during infection by *South African cassava mosaic virus* of susceptible (T200) and tolerant (TME3) cassava landraces. Abundant SEGS-derived small RNAs were also present in mock-inoculated and *South African cassava mosaic virus*–infected T200 and TME3 leaves. Given the known role of transposable elements, and 24-nt RNAs targeting transposable elements, in gene regulation and plant immune responses, our observations are consistent with a role for these DNA elements in the host's regulatory response to geminiviruses. The ability of SEGS1 to overcome *CMD2* tolerance and the transmission of SEGS2 by whiteflies has potential implications for the long-term durability of *CMD2* tolerance that has been widely deployed in Africa (6).

Cassava mosaic viruses are transmitted by distinct *Bemisia tabaci* haplotypes. *B. tabaci* is now known to be a complex consisting of more than 35 morphologically indistinguishable species (61) divided into distinct clades (62). Coadaptation between cassava mosaic geminiviruses and their local vector populations has been proposed (63). More recently, sub-Saharan Africa was shown to harbor mainly noninvasive indigenous *B. tabaci* types (clustering into five subgroups, named SSAF-1–5) that presumably serve as the natural vectors of the continent's many indigenous begomovirus species (54, 55, 64–66). SSAF-1 is now known to be the most widely established group of whiteflies associated with cassava in Africa and is likely the primary vector of the various cassava mosaic geminivirus species that are distributed throughout the continent (54, 55, 64–66). The SSAF-2 subclade includes whiteflies from Uganda described previously as the invasive Uganda U2 population, which has been associated in the past two decades with extremely severe CMD epidemics (mixed ACMV and EACMV-Uganda infection) (39) in East and Central Africa (51, 67). In addition to these indigenous *B. tabaci* types, introduced nonindigenous B and Q types have been reported in a number of regions in sub-Saharan Africa (54, 55, 66) and could potentially be an important driver for cassava mosaic geminivirus emergence in the future.

Cassava Brown Streak Disease

Over the past decade, CBSD has emerged as a risk to food security and a major threat to the cassava value chain due to the lower quality and palatability of storage roots from cassava plants carrying this disease. In this section, we report on the discovery of the causative viruses, as well as their epidemiology and diversity.

First reports. The presence of a second disease, initially termed brown streak, was first recognized in 1935 by a researcher from the Amani Research Station in Tanganyika Territory, now known as Tanzania (68). Originally recorded in the foothills of the Usambara Mountains, CBSD was later reported to be widespread at low altitudes, including in coastal areas where cassava is grown (69). An early report of CBSD also mentions the impact of environmental conditions on symptom expression in diseased plants (68). Until recently, CBSD was restricted to low-altitude regions of East Africa (70). Soon after the first report of CBSD, whitefly was identified as a possible vector of

the disease (71). CBSD remained largely ignored until its prevalence increased and its impact on cassava production became more prominent in Malawi, Mozambique, and Tanzania in the 1990s.

The causal agent: discovery of *Cassava brown streak virus*. In the first report of the disease, it was hypothesized that the causal agent might be a virus (68). Despite the rapid characterization of the viral nature of the disease by sap transmission to herbaceous hosts (72), the virus was not identified until the coat protein was first sequenced (73). The virus was subsequently named *Cassava brown streak virus* and was classified in the genus *Ipomovirus*, family *Potyviridae*. Nearly a decade later, its genome was fully sequenced and its genomic structure confirmed to be closely related to other ipomoviruses (74). CBSD is now known to be caused by two distinct but closely related virus species: *Cassava brown streak virus* and *Ugandan cassava brown streak virus* (11, 75). Virions of both species are flexuous particles 650 nm in length with positive-sense ssRNA genomes (**Figure 3*b***). Like other ipomoviruses, cassava ipomoviruses have a single, long ORF encoding a polyprotein that is processed into 10 predicted proteins (**Figure 3*b***) by cleavage at conserved sites (76). They are distinct from other ipomoviruses in that they have a single P1 protein, which suppresses RNA silencing, and lack an HC-Pro protein. In addition, they also encode a predicted HAM1h protein that is located between NIb and CP on the polyprotein. This HAM protein shares conserved amino acids with Maf/HAM proteins, which are nucleoside triphosphate pyrophosphatases involved in reducing the mutation rate (74). A short overlapping ORF named pretty interesting *Potyviridae* ORF (PIPO) (77) lies in the *P3* coding sequence of the genome. Its requirement for infection has not yet been demonstrated.

Epidemiology. The initial observation that CBSD incidence is higher in coastal areas (69) was later confirmed by independent studies (78, 79). Both cold temperature and lack of potential vectors at altitudes above 1,000 m above sea level were considered as factors contributing to the absence of CBSD at higher altitudes. *Bemisia afer* (Priesner-Hosny) was initially hypothesized to be the vector of cassava ipomoviruses because of its presence predominantly on lower leaves of cassava, where CBSD symptoms are more prominent (78). Experiments with *B. afer* (Priesner-Hosny) and *B. tabaci* (Gennadius) under controlled conditions have subsequently shown that only *B. tabaci* (Gennadius) can vector cassava ipomoviruses. Their transmission by *B. tabaci* appears to be semipersistent (14).

The outbreak of CBSD around the Great Lakes region in 2004 (70, 80) prompted the research community to investigate the factors involved in dissemination and pandemics of CBSD. Diversity and virulence studies suggested that the CBSD outbreak was not driven by the appearance of new virulent strains in the Great Lakes region (9, 75). Superabundant populations of *B. tabaci* appeared to coincide with high incidence of CBSD in several regions (67), and it has been assumed that they drove the expansion of CBSD to nonaffected areas because their abundance often increases prior to CBSD pandemics. Other driving forces might be at stake in dissemination of cassava ipomoviruses. For example, the contribution of cassava ipomovirus infection to the attraction of *B. tabaci* by the cassava host should be further studied, as plant viruses have recently been shown to manipulate plants to attract vectors (81, 82). Recent studies have also shown that minimum temperature remains the most important factor for CBSD incidence in both the coastal and Great Lakes regions (80). However, low temperatures have been associated with reduced RNA silencing antiviral defense and high viral replication in other pathosystems (83, 84). Field observations suggest that high temperatures tend to reduce CBSD symptoms in cassava (85), but studies under controlled conditions are required to better understand the role of minimum temperature in CBSD incidence. Because CBSD incidence primarily reflects transmission of the virus, temperature should also be further investigated for its potential impact on virus transmission by vectors.

Importantly, the human factor should not be underestimated in the dissemination of CBSD, and programs aimed at wide distribution of planting material to farmers have now integrated diagnostics components in order to prevent dissemination of cassava ipomoviruses across regions and borders by transport of planting material (**Table 1**).

Diversity. Initial diversity studies identified *Cassava brown streak virus* and *Ugandan cassava brown streak virus* as the two species associated with CBSD (11, 75, 86). Although they were primarily detected in coastal areas of Tanzania and in Uganda, respectively, surveys have shown that both species co-occur in the CBSD-affected regions of Kenya, Malawi, Uganda, and Tanzania (86).

Table 1 A nonexhaustive list of projects ongoing and carried out in the past decade aimed at limiting cassava losses due to viral diseases on the African continent

Project name	Virus-related objectives	Operating countries	Period	Funding
BioCassava Plus Phase 1	Development of virus-resistant cassava through genetic engineering	Nigeria, Kenya	2005–2010	BMGF
Virus Resistant Cassava for Africa (VIRCA)	Development of virus-resistant cassava through genetic engineering	Uganda, Kenya, Nigeria	2006–2022	BMGF, USAID, and Monsanto Fund
Great Lakes Cassava Initiative (GLCI)	Cooperative research into new disease-resistant varieties and surveillance	Uganda, DRC, Tanzania, Kenya, Burundi, Rwanda	2008–2012	BMGF
LimitCBSD	CBSD diagnostics, epidemiology, and tissue culture and use of advanced molecular tools to identify sources of resistance to the disease	Malawi, Kenya, Tanzania	2012–2016	African Union
Community Action in Controlling Cassava Brown Streak Disease Control Through Clean Seed (Community Phytosanitation)	Distribution of clean seed and use of virus-resistant varieties	Tanzania	2012–2016	BMGF
Southern African Value Added Cassava (SAVUCA)	Development of virus-resistant cassava through genetic engineering and investigation of molecular determinants for natural virus resistance/tolerance	South Africa	2013–2017	SNF and NRF
Disease Diagnostics for Sustainable Cassava Productivity in Africa	Development and implementation of tools for disease monitoring in cassava fields	Kenya, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zambia	2013–2017	BMGF and DFID
Indo-Swiss Cassava Network	Development of virus- and whitefly-resistant cassava through genetic engineering and conventional breeding	India	2014–2019	SDC and DBT
Building a Sustainable, Integrated Seed System for Cassava in Nigeria (BASICS)	Development of a cassava seed system for distribution of disease-free planting material	Nigeria	2015–2019	BMGF

(*Continued*)

Table 1 (*Continued*)

Project name	Virus-related objectives	Operating countries	Period	Funding
Building an Economically Sustainable Seed System for Cassava in Tanzania (BEST Cassava)	Distribution of clean seed and use of virus-resistant varieties	Tanzania	2017–2022	BMGF
Fighting Cassava Brown Streak Disease and Cassava Mosaic Disease	Generation of virus-resistant cassava through conventional breeding and distribution of clean seeds	Rwanda, Burundi	2017–2021	IFAD
New Cassava Varieties and Clean Seed to Combat CBSD and CMD (5CP)	Distribution of clean seed and use of virus-resistant varieties	Malawi, Mozambique, Kenya, Uganda, Tanzania	2012–2017	BMGF
Biotechnology Applications to Combat CBSD	Generation of virus-resistant cassava through marker-assisted breeding	Tanzania, Uganda	2011–2016	BMGF
West Africa Virus Epidemiology (WAVE)	Epidemiology of cassava viral disease in West Africa; production and distribution of disease-free planting material	Benin, Burkina Faso, Ivory Coast, Ghana, Nigeria, Togo	2015–2018	BMGF

Abbreviations: BMGF, Bill & Melinda Gates Foundation; CBSD, cassava brown streak disease; DBT, Department of Biotechnology; DFID, Department for International Development; DRC, Democratic Republic of Congo; IFAD, International Fund for Agricultural Development; NRF, National Research Foundation (South Africa); SDC, Swiss Agency for Development and Cooperation; SNF, Swiss National Research Foundation.

Recent studies using whole-genome sequences have confirmed the presence of two primary viral clades [i.e., cassava brown streak viruses (CBSVs) and Ugandan cassava brown streak viruses (UCBSVs)], subdivided into additional clades (86–88). UCBSV species appear to encompass more clades as compared with CBSV species, but CBSV genomes are genetically more diverse; the evolution rate of CBSVs is estimated to be five times higher than that of UCBSVs (88). Notably, *NIa*, *6K2*, *NIb* and *P1* (**Figure 3*b***) are the CBSV sequences contributing to this accelerated evolution rate. Although high-throughput sequencing technologies have permitted rapid progress in the characterization of virus diversity, it is important to note that the recently reported full genomes of *Cassava brown streak virus* and *Ugandan cassava brown streak virus* were generated by de novo assembly of relatively short reads (87, 88). Ultimately, reverse transcription polymerase chain reaction amplification and sequencing of amplicons would be necessary to validate the true viral genomes present in the host (89). Characterizing the diversity of virus isolates encountered in the field is critical to the assessment and development of varieties with broad-spectrum resistance. CBSD resistance can be considered as broad-spectrum only once the resistant cassava lines have been tested against a selection of genetically diverse CBSV and UCBSV isolates.

CONTROL AND MANAGEMENT OF CASSAVA MOSAIC AND BROWN STREAK DISEASES: THE WAY FORWARD

The majority of cassava growers in Africa are subsistence or small-scale farmers, so ongoing effective traditional control strategies are critical for reducing CMD and CBSD incidence. These strategies were recently comprehensively reviewed by Legg et al. (7). The exchange between and within countries of cassava germplasm by nodal stems has exacerbated the risk of virus spread. The emergence of CBSD in the Great Lakes region of East/Central Africa (6) and the introduction

of *East African cassava mosaic virus* isolates into the Indian Ocean islands (90) are recent examples of propagation of infected plant material. The drawback of tissue culture–mediated virus-free planting material or synthetic seeds and somatic embryos (91) resides in the genotype dependency and induced epigenetic variation intrinsic to in vitro culture. Additionally, propagation by nodes in vitro has recently been shown to alter the methylation pattern of plants when compared with field stakes (92). Despite numerous conventional breeding efforts over the past few decades for resistance to CBSD and CMD in Africa and Asia (7, 93–100), these two diseases remain major contributors to cassava yield loss, and new technological approaches (91) need to be adopted and improved. Although biotechnology has been used extensively as a tool to develop cassava resistant to CMD and CBSD (101–104), genetically modified varieties have not yet been released in Africa or Southeast Asia. The longer-term goal is to combine high levels of resistance to CMD, CBSD, and whitefly through either gene editing or transgenic approaches. The loss of *CMD2* resistance (105) to cassava disease in plants generated through somatic embryogenesis has also prompted the urgent need to develop transformation methods that do not alter *CMD2*-based resistance.

Natural Resistance to Cassava Mosaic and Brown Streak Diseases

Recently, some advances in our understanding of mechanisms associated with natural virus resistance genes have been made. We review here the current status of knowledge about dominant and recessive resistance genes in cassava, which could be deployed in future breeding programs for resistance against CMD and CBSD.

Molecular breeding. Genetic improvement through conventional breeding in cassava is a challenging and lengthy process. An early milestone in molecular breeding was the identification of simple sequence repeat and restriction fragment length polymorphism molecular markers associated with the putative single dominant resistance gene *CMD2* (90, 91). These markers were initially used to introgress the *CMD2* locus into Latin American cultivars for deployment in Africa (92). A more recent high-density single-nucleotide polymorphism map of cassava using genotype-by-sequencing revealed a single putative chromosomal location of *CMD2*-associated dominant resistance (93). Two other sources of resistance have also been identified: polygenic recessive resistance (*CMD1*) (94) derived from *Manihot glaziovii* and, more recently, the *CMD3* locus (95), which was recently suggested to colocalize with *CMD2* on chromosome 8 (96). Significantly, Wolfe et al. (96) also identified 13 other regions of small effect, including one on chromosome 9 that colocates with *CMD1*. Molecular markers have also been identified for CBSD tolerance and resistance in local African cultivars using biparental quantitative trait locus mapping (7).

A notable advance in the past two decades was the release of the draft genome sequence of a partial inbred cassava line, AM560, in 2009 (97). Most recently, extensive whole-genome shotgun sequencing of many wild and cultivated *Manihot* species has revealed possible sources for virus resistance (98); for example, some CBSD-resistant cassava varieties in Tanzania, including Namikonga and Muzege, contain sections of genomes of *M. glaziovii*. Tolerance to CMD (99) and CBSD derived from several cassava landraces and wild *Manihot* species (98, 146, 147) could be exploited further in breeding programs.

In pursuit of the identification of immunity-related or resistance genes. Using a combination of genotype-by-sequencing-based single-nucleotide polymorphisms and physical mapping of scaffolds from cassava whole-genome sequencing, 1,061 cassava immunity-related genes were mapped (100). Notably, from a list of 105 putative *CMD2* genes identified from the *CMD2* locus on chromosome 8 (96), 35 were identical to those identified in an RNA sequencing study of *South*

African cassava mosaic virus–infected TME3 (101) and could be strong candidates contributing to resistance or tolerance in cassava. Resistance (R) gene–encoded proteins usually exist as large families of proteins with nucleotide-binding site (NBS)–leucine-rich repeat (LRR) domains and function as indirect sensors of pathogen avirulence proteins. The determinants for apparent virus R gene–wide specificity lies in the LRR domains, and sequencing of wild cassava varieties may provide a source for discovery of new resistance to CMD and CBSD. Recently, 228 NBS-LRR and 99 partial NBS genes were mapped to the cassava reference genome; they show high sequence similarity to R genes from other plant species (102), but their roles in CMD or CBSD resistance are not known. RNA sequencing analysis of NBS-LRR gene expression in response to cassava ipomovirus infection in Kaleso (resistant) and Albert (susceptible) cassava genotypes showed no differential expression of NBS-LRR genes one year after infection (103). However, it cannot be ruled out that the proteins encoded by these genes could function in virus recognition earlier in the infection process. Alternatively, phloem-associated non-NBS-LRR proteins, such as jacalin or small heat shock proteins, could be promising candidates against cassava ipomoviruses because these viruses locate to both roots and leaves. These proteins have been found to restrict long-distance movement of the potyvirus *Tobacco etch virus* (104).

Leal et al. (105) identified predicted immunity-related gene pathways or networks in cassava, and further studies on resistance or immunity gene function and evolution from cassava genotypes could positively influence the development of durable resistance to cassava viruses. The recent finding that decoy engineering can expand the recognition specificity of plant immune receptors, such as NBS-LRRs, opens a wealth of opportunities for resistance breeding (106). It was recently shown that novel recognition specificities of plant immune receptors to unrelated effectors can be engineered (106). Identified cassava immune receptors could be engineered to exhibit effector-triggered immunity against cassava viruses. Durable nonhost resistance is not known in cassava, and its mechanisms are poorly understood, but nonhost resistance is likely to be an intrinsic lack of susceptibility and a multigenic trait (107). Recessive resistance linked to impaired susceptibility to potyvirus infection has been shown to be due to the lack of or mutation in one of the eukaryotic translation initiation factors 4E and 4G (107, 108) and could therefore potentially be valuable to exploit for cassava resistance to cassava ipomoviruses in the future. Further work is required to determine whether disruption of the interaction between cassava ipomovirus VPg and cassava translation initiation factors could confer broad-spectrum resistance to these viruses in cassava.

Exploiting functional genomics, host-pathogen interactions, and proteomics to improve cisgenic resistance. Functional genomics offers the ability to identify natural resistance or immunity genes involved in plant responses to virus infection. Cisgenesis is genetic modification to transfer beneficial alleles or genes from crossable species into a recipient plant (109), and it offers potential new applications in plant breeding for CMD and CBSD. Currently, new genome editing tools, such as zinc finger nucleases, transcription activator–like (TAL) effector nucleases, and the clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated protein (Cas) system (110, 111), represent opportunities for generating modified cassava that could bypass cumbersome regulation of transgenics. TALs offer a new approach to exploit plant-pathogen interactions to engineer CMD or CBSD resistance; these proteins are able to bind to host DNA promoter regions (effector-binding elements, or EBEs) in a base-specific manner and manipulate transcriptional activity of target susceptibility (S) genes (111). Another approach would be to remove or modify natural EBE boxes from S gene promoters in cassava or design and add EBEs as molecular traps for activation of executor R genes (112). Although in cassava no

executor R genes have been identified, new sources of R genes have recently been reported (100, 102).

Geminiviruses induce changes in their host cells by manipulating host molecular pathways (reviewed in 30). Cassava mosaic geminiviruses (101) and cassava ipomoviruses (103, 147) induce global transcriptome reprogramming of cassava. An understanding of the roles of host reprogramming (101) and RNA silencing (small interfering RNA, or siRNA, responses) during cassava-virus interactions (113, 114) could also be exploited to improve natural immunity. Protein-protein interaction technologies and translational proteomics can also reveal potential solutions to the discovery of resistance factors in both model and nonmodel host plants (115). Proteomic studies extended to virus-host interactions, such as interactions of cassava viruses with cassava, can provide insights into virus pathogenicity determinants and host responses and inform development strategies for cassava virus resistance. A better understanding of the multifactorial nature of cassava mosaic and brown streak virus resistance is challenging and will require deciphering complex networks that underpin host-pathogen interactions (116, 147). Future computational developments in applying network modeling algorithms will be critical in identifying the molecular mechanisms underlying CMD and CBSD.

Engineered Resistance to Cassava Mosaic and Brown Streak Diseases

Transgenic technologies offer an alternative approach to molecular or conventional breeding for introducing virus resistance traits into cassava. These technologies are invaluable for the development of virus-resistant farmer-preferred cultivars that are restricted by limitations inherent to traditional breeding.

Transgenes expressing dsRNAs. The RNA silencing pathway has become a powerful tool for engineering resistant plants (117). The most efficient approach to RNA interference (RNAi) is the expression of a transgene-expressed hairpin RNA that generates siRNAs targeting virus genes. Early transgenic resistance to CMD involved expression of antisense RNA targeting the ACMV-Kenya *Rep*, *TrAP*, and *Ren* ORFs (118). It was later demonstrated that expression of intron-containing hairpin/dsRNA homologous to the bidirectional promoter region of ACMV-Kenya DNA-A confers improved recovery from infection in transgenic cassava (119). The most robust resistance using intron-containing hairpin/dsRNA has been achieved by targeting the *African cassava mosaic virus AC1* sequence (120). Based on the evidence that the dsDNA phase of geminiviruses is prone to methylation by the silencing machinery, the resistance conferred by targeting geminivirus coding sequences is hypothesized to rely on posttranscriptional gene silencing of viral transcripts as well as transcriptional gene silencing through methylation of the geminivirus genome (121). RNAi-mediated resistance has also been achieved against *Ugandan cassava brown streak virus* (122) as well as against both *Cassava brown streak virus* and *Ugandan cassava brown streak virus* by targeting coat protein sequences (123). Ongoing field assessments of transgenic cassava resistant to cassava ipomoviruses suggest that the genetically engineered CBSD resistance is stable over several vegetative cropping cycles and in diverse agroecological locations (124). It can be concluded from independent lab and field experiments that expression of hairpin RNAs in transgenic cassava does not lead to transgene silencing over time and multiplication cycles, which is a prerequisite for the deployment of virus-resistant transgenic cassava in the field. Given the high mutation rates of RNA viruses (125) and the reliance of RNAi-based resistance on sequence homology between hairpin-derived siRNAs and target viral sequences, deployment of transgenic resistant lines will certainly require regular monitoring of virus sequence diversity in the field.

Improving transformation and regeneration of model cassava cultivar 60444 and farmer- and industry-preferred high-yielding landraces. From the first early reports of efficient cassava transformation and regeneration (126, 127) to more recent improved methods (128), the application of cassava transformation of friable embryogenic callus in virus resistance and other traits remains critically important (129, 130). There is a critical need to rapidly transfer genetic transformation capacities from the model cultivar 60444, routinely used for proof-of-concept research in virus resistance, to farmer- and industry-preferred cultivars. Successful transformation and regeneration of African farmer- and industry-preferred landraces such as TME3, TME7, TME204, T200, Ebwanatereka, Kibandameno, and Serere have been reported (123, 131–134). Efforts have also been made to effectively transfer cassava genetic transformation technologies to laboratories located in cassava-growing regions (135). Despite recent successful friable embryogenic callus–based genetic transformation of the aforementioned cultivars, production of transgenic cassava remains challenging as transformation and regeneration efficiencies continue to be low and genotype dependent. Recent studies also indicate that embryogenesis and meristem subculturing lead to important changes in gene expression and epigenetic marks (136, 137). The loss of *CMD2* resistance during embryogenesis calls for better characterization of those transcriptional and epigenetic changes as well as for the development of transformation methods that minimize those changes.

Bemisia tabaci Control

Control of whiteflies (138) would reduce the spread and impact of cassava geminiviruses and ipomoviruses. A recent study reports the identification of the Tma12 protein from an edible fern [*Tectaria macrodonta* (Fée) C. Chr.] that is insecticidal to whitefly and confers whitefly resistance in cotton by interfering with the whitefly life cycle at sublethal doses (139). This strategy may be well suited for deployment in genetically modified cassava to control *B. tabaci* and the transmission of cassava mosaic geminiviruses and cassava brown streak viruses. Studies on natural enemies of *B. tabaci* have identified at least nine parasites, and mycoinsecticide products have been derived from three fungal species: *Verticillium lecanii*, *Paecilomyces fumosoroseus*, and *Beauveria bassiana*. However, usage has been limited by cost, shelf life, and dependence on humid and warm environmental conditions; farmers prefer the relatively straightforward application of more toxic nonbiological insecticides (140).

Although the use of pesticides has helped with managing whiteflies in the field, there is an increasing need to develop novel biotechnological strategies, such as RNAi technology, that are effective at controlling whitefly populations (141). Oral delivery of dsRNA targeting actin ortholog, ADP/ATP translocase, α tubulin, ribosomal protein L9, and v-ATPaseA was shown to cause different degrees of whitefly mortality (142). The efficacy of exogenous dsRNA application is highly dependent on the vehicle used to deliver the dsRNA to target cells (143). Some predicted potential drawbacks include incomplete protection of crops owing to the high LC_{50} values required, toxicity of the insecticidal molecules to nontarget insects, and physiological adaptations in target pests. Alternatively, RNAi can be used to target important whitefly genes via transgene-dsRNA expression. For example, whitefly resistance was achieved by genetic transformation of tobacco, which generated siRNA against the whitefly v-ATPaseA gene (144). Recent reports suggest that expression of dsRNA targeting acetylcholinesterase and ecdysone receptor confers whitefly resistance in transgenic tobacco (144).

Elucidation of the molecular mechanisms and metabolic pathways of plant defense against whitefly is paramount in developing control strategies. Few studies have been performed in this regard, but differentially expressed genes, including chitinases, lipoxygenases, and caffeoyl-CoA

O-methyltransferase (involved in lignin biosynthesis), were induced in the whitefly-resistant cassava landrace Ecu72 when it was exposed to *Aleurotrachelus socialis* (129). Preliminary screening assays suggested that the whitefly resistance of Ecu72 also holds against *B. tabaci* (145), opening new opportunities for identification of molecular pathways associated with *B. tabaci* resistance in cassava as well as for rapid introgression into cassava cultivated in CMD- and CBSD-endemic regions.

SIGNIFICANT GLOBAL PARTNERSHIPS AND INITIATIVES FOR MANAGEMENT OF CASSAVA VIRUS DISEASES AND THEIR WHITEFLY VECTOR

Several partnerships and initiatives over the past decade have been established to develop new molecular and genetic tools to assist breeders in creating higher-yielding, more nutritious cassava with resistance to pests and diseases. Highlights of global initiatives contributing to cassava virus and whitefly resistance improvement are summarized in **Table 1**. These initiatives often rely on the development of diagnostic tools for CMD and CBSD and the distribution of disease-free seeds. Although immediate impacts have been recorded following the distribution of virus-free planting material, the long-term establishment of a seed system for cassava remains challenging. In the future, commonalities with potato, a vegetatively propagated crop with a well-established seed system in the Northern Hemisphere, should be further exploited for the large-scale production and distribution of disease-free cassava seeds in Africa.

CONCLUDING REMARKS

Several milestones over the past two decades have been achieved in the elucidation of cassava virus biology, structure, and function. Remarkable progress in high-throughput sequencing techniques, functional genomics, and proteomics has contributed to insights into cassava virus interactions with natural and experimental hosts that can be exploited to enhance natural resistance. Advances in cassava transformation and regeneration open up opportunities to engineer RNAi-mediated virus resistance, not only in model cultivar 60444 but in other cassava varieties and landraces as well. Virus resistance can be stacked with other traits, such as nutritional and yield enhancement. In spite of these benefits, genetically modified crops have aroused opposition, and fears regarding health and environmental risks need to be allayed by science-based risk assessments of transgenic cassava and by additional field-generated data. Other challenges need to be addressed before the reality of virus-free cassava cultivation can be achieved, such as access to virus-free germplasm and development of a reliable seed system; establishment of functional regulatory and biosafety processes in many cassava-growing countries; deployment of improved virus-resistant varieties; continuous monitoring of germplasm; and applications of improved technologies. Recent progress in sequencing and genome editing technologies offers unprecedented opportunities to investigate and exploit natural resistance against cassava viruses. Although losses due to diseases can be rapidly reduced by investment in applied research and disease-free dissemination programs, long-term solutions to cassava diseases will not materialize without a better understanding of cassava-virus-vector interactions.

SUMMARY POINTS

1. Molecular methods and next-generation sequencing have significantly advanced our understanding of cassava virus diversity and genome molecular functions, especially within the past decade.

2. Whole-genome sequences have confirmed the presence of two primary viral clades (CBSVs and UCBSVs) responsible for CBSD; these are subdivided into additional clades. Notably, the *NIa*, *6K2*, *NIb* and *P1* genomic sequences contribute to the accelerated evolution rate of CBSVs.
3. Transmission of cassava brown streak and mosaic viruses by several sub-Saharan African indigenous *B. tabaci* genotypes has been demonstrated.
4. Genetic modification technologies and improvements in cassava transformation have led to successful engineering of resistance to cassava brown streak and mosaic viruses.
5. Molecular mapping and sequencing of the cassava genome has contributed significantly to identifying candidate gene loci associated with CMD tolerance.

FUTURE ISSUES

1. How cassava virus diversity is generated in the field and how genotypes with enhanced pathogenicity establish themselves remain to be understood.
2. More research into the genetic diversity and taxonomy of *B. tabaci* and the transmission of cassava brown streak and mosaic viruses will be invaluable.
3. The molecular networks involved in susceptibility or resistance to cassava viruses require further elucidation.
4. Further research into the tripartite relationship between cassava brown streak and mosaic viruses, the whitefly vector, and the cassava host—and the impact of this relationship on the epidemiology of these diseases—is critical.
5. Exploitation of natural resistance in cassava, and applications of new technologies such as gene editing, will complement genetic modification approaches.

DISCLOSURE STATEMENT

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