Review

Cassava brown streak disease: a threat to food security in Africa

Basavaprabhu L. Patil,[1] James P. Legg,[2] Edward Kanju[2] and Claude M. Fauquet[3]

Correspondence
Basavaprabhu L. Patil
blpatil2046@gmail.com
Claude M. Fauquet
c.fauquet@cgiar.org

[1]National Research Centre on Plant Biotechnology, IARI, Pusa Campus, New Delhi 110012, India

[2]International Institute of Tropical Agriculture, PO Box 34441, Dar-Es-Salaam, Tanzania

[3]Centro Internacional de Agricultura Tropical, Apartado Aéreo 6713, Cali, Colombia

Cassava brown streak disease (CBSD) has emerged as the most important viral disease of cassava (*Manihot esculenta*) in Africa and is a major threat to food security. CBSD is caused by two distinct species of ipomoviruses, *Cassava brown streak virus* and *Ugandan cassava brown streak virus*, belonging to the family *Potyviridae*. Previously, CBSD was reported only from the coastal lowlands of East Africa, but recently it has begun to spread as an epidemic throughout the Great Lakes region of East and Central Africa. This new spread represents a major threat to the cassava-growing regions of West Africa. CBSD-resistant cassava cultivars are being developed through breeding, and transgenic RNA interference-derived field resistance to CBSD has also been demonstrated. This review aims to provide a summary of the most important studies on the aetiology, epidemiology and control of CBSD and to highlight key research areas that need prioritization.

Cassava and viral diseases of cassava

Cassava (*Manihot esculenta* Crantz, family Euphorbiaceae), which produces carbohydrate-rich tuberous roots, is an important staple food crop for about 800 million people across the globe, and is cultivated mostly as a subsistence crop but also for its industrial value (Thresh, 2006). Cassava is vulnerable to at least 20 different viruses, of which those causing cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are the two most economically important, resulting in production losses over US$1 billion every year (Legg *et al.*, 2006; IITA, 2014). CMD is caused by cassava mosaic geminiviruses (CMGs) (Bock & Woods, 1983; Hong *et al.*, 1993), which are circular ssDNA viruses that been studied extensively since the 1990s (Legg & Fauquet, 2004; Legg *et al.*, 2011; Patil & Fauquet, 2009). Contrastingly, CBSD is caused by cassava brown streak viruses (CBSVs) (Monger *et al.*, 2001; Winter *et al.*, 2010), which are positive-sense ssRNA viruses. CBSD was first reported from the coastal region of Tanzania in the 1930s (Storey, 1936) but has received much less attention than CMD, partly due to its earlier geographical restriction to lowland areas of East Africa (Hillocks & Jennings, 2003; Hillocks *et al.*, 1999; Nichols, 1950). However, since 2004, this situation has changed and CBSD has been spreading at an alarming rate in East and Central Africa, threatening the food security of millions of cassava farmers (Alicai *et al.*, 2007; Pennisi, 2010; Legg *et al.*, 2011, 2014a).

Aetiology, transmission, host range and diagnosis of CBSD

CBSD affects cassava, and no alternative crop or weed hosts have been reported, although there has been a recent report on the detection of CBSV in the wild cassava relative *Manihot glaziovii* (Mbanzibwa *et al.*, 2011a). Two species of ipomovirus are known to cause CBSD: Cassava brown streak virus (Monger *et al.*, 2001) and Ugandan cassava brown streak virus (Mbanzibwa *et al.*, 2009a). Ugandan cassava brown streak virus (UCBSV) was initially referred as to as cassava brown streak Uganda virus (Patil *et al.*, 2011). Here, we use the general term CBSVs when referring to both viruses. The aerial symptoms of CBSD in cassava include feathery chlorosis along the veins of the leaves or sometimes circular patches of chlorosis in between the primary veins, brown necrotic streaks on the stem and stem die-back in severe cases (Fig. 1a–c) (Jennings, 2003; Nichols, 1950). Symptoms in the tuberous roots consist of a brown, corky necrosis of the starchy tissue, occasional radial constrictions and a reduction in the content of starch and cyanide (Fig. 1d, e) (Hillocks & Jennings, 2003; Nichols, 1950). The viral symptom phenotypes are variable depending on the virus isolate involved, variety of cassava, age of plant and the environmental conditions (Patil & Fauquet, 2014). In a recent study, Mohammed *et al.* (2012) characterized the symptoms produced by different isolates of CBSV and UCBSV, in both cassava and *Nicotiana*
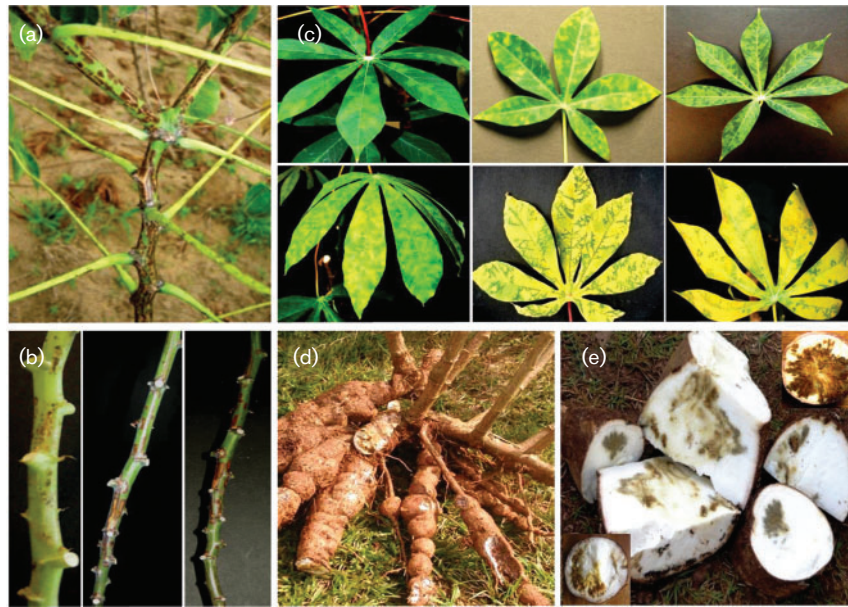
Fig. 1. CBSD symptoms visible on different parts of the cassava plant. (a, b). Brown streaks on the young stem. (c) Different levels of feathery chlorosis on leaves. (d) Constrictions on the root surface. (e) Root necrosis.

benthamiana, under uniform conditions and identified contrasting levels of symptom severity produced by different isolates. Several herbaceous plant species of different families can be artificially infected using mechanical transmission methods, and the variation in symptom phenotypes is more pronounced in the model host *N. benthamiana* (Lister, 1959; Mbanzibwa *et al.*, 2009b; Mohammed *et al.*, 2012; Ogwok *et al.*, 2010; Winter *et al.*, 2010). Comparison of symptom severity between isolates of CBSV and UCBSV has shown that generally CBSV causes more severe symptoms, causing necrosis in *N. benthamiana*, whereas with UCBSV only mosaics and rugosity are induced in this host (Mbanzibwa *et al.*, 2011b; Mohammed *et al.*, 2012; Winter *et al.*, 2010). Grafting experiments in cassava cultivars also demonstrated higher virulence of CBSV than UCBSV, and the cuttings infected with CBSV showed significantly reduced sprouting, as a result of higher virus accumulation, compared with UCBSV (Mohammed *et al.*, 2012; Wagaba *et al.*, 2013). Under artificial conditions in *N. benthamiana*, UCBSV and CMGs interact synergistically (Ogwok *et al.*, 2010); however, there are no reports of synergism in field-grown cassava.

The whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), was proposed to be the vector of CBSVs as early as the 1930s (Storey & Nichols, 1938), but this was not confirmed until many years later (Maruthi *et al.*, 2005). Unlike CMGs, which are transmitted by *B. tabaci* in a persistent manner, CBSVs are transmitted semi-persistently, like other ipomoviruses, and are not retained for more than 24 h (Dombrovsky *et al.*, 2014; Jeremiah, 2014; M. Maruthi, unpublished data).

Early and accurate diagnosis of CBSVs in diseased plants remains a great challenge. Effective diagnostics will help to monitor and forecast disease outbreaks, giving enough time for the application of management strategies (Martin *et al.*, 2000; Miller *et al.*, 2009). There are several methods available for detection and diagnosis of CBSD-causing viruses. Using ELISAs, it is possible to detect CBSD but not to distinguish between CBSV and UCBSV (Winter *et al.*, 2010). Several nucleic acid-based methods have been employed for diagnostics of CBSD viruses, such as reverse transcription (RT)-PCR (Abarshi *et al.*, 2010, 2012; Mbanzibwa *et al.*, 2011a; Moreno *et al.*, 2011), real-time RT-PCR (Adams *et al.*, 2013) and more recently loop-mediated isothermal amplification (Tomlinson *et al.*, 2013). These PCR-based approaches allow specific detection of CBSV and UCBSV, and when used with multiplexing primers, they may specifically and differentially amplify the target regions of CBSV and UCBSV (Abarshi *et al.*, 2012). With advances in sequencing technologies, hitherto unknown viruses are now being identified by deep sequencing of RNA extracts from virus-infected plants (Kreuze *et al.*, 2009). This approach has also been used for diagnosis of CBSVs (Monger *et al.*, 2010).

Current status of CBSD in Africa and effects on yield

The earliest report of CBSD was from northern coastal areas of Tanzania in 1935 (Storey, 1936), corresponding to the region where CMD was first observed (Warburg, 1894) and emphasizing the fact that Tanzania is a hot spot for biodiversity of cassava viruses (Ndunguru *et al.*, 2005). Early reports of CBSD noted that affected areas were almost entirely restricted to coastal areas of East Africa and

the shores of Lake Malawi (Nichols, 1950). Although CBSD was observed in some parts of Uganda, for many years it was believed that the disease did not spread at altitudes over 1000 m above sea level (a.s.l.) (Nichols, 1950).

The first systematic countrywide assessment of CBSD was completed in 1994 in Tanzania (Legg & Raya, 1998) and the highest incidences were recorded from the southern lowland coastal districts of Mtwara (36.0 %) and Masasi (25.2 %), whilst the disease was virtually absent from the mid-altitude (>800 m a.s.l.) region of north-western Tanzania. Significantly, however, small numbers of symptomatic plants were observed near Entebbe, in central/southern Uganda (~1200 m a.s.l.) in 1994 (J. M. Thresh, unpublished data) and from Tabora in north-western Tanzania (~1200 m a.s.l.) (Legg & Raya, 1998). However, the view that CBSD is a lowland disease remained unchanged until 2004, when the first report was made of significant spread of CBSD in central/southern Uganda (Alicai et al., 2007). Following these first reports from Mukono district, significant increases in the incidence and distribution of the disease were recorded in Uganda through to 2007, by which time approximately 10 % of all fields included infected plants and overall incidence was 1.9 % (Alicai et al., 2007). More recent estimates, obtained by Uganda's National Agricultural Research Organization, put the overall incidence at 16 % in 2008 and 29 % in 2009 (T. Alicai, unpublished data), clearly illustrating the rapid expansion of the new CBSD epidemic. Shortly after CBSD was reported to be spreading in Uganda, similar observations were made in western Kenya (Ntawuruhunga & Legg, 2007) and the Lake Victoria zone of Tanzania. The incidence of CBSD increased in Tanzania in a similar manner, and surveys of 19 districts within the north-western regions of Kagera, Mara, Shinyanga and Kigoma revealed a steady pattern of increase in CBSD incidence from 5.9 % in 2006 to 11.5 % in 2007 and 31.6 % in 2008 (Legg et al., 2011; I. Ndyetabula, unpublished data). Most of the increase was attributed to greater levels of disease in districts in which CBSD was already present by 2006 (12 of 19 districts; Legg et al., 2011). All of these survey assessments, based primarily on visual assessments of leaf symptoms, are underestimates of the true level of infection, as CBSD leaf symptoms may not be expressed where weather conditions are unfavourable. Additional reports have also been published in recent years from Rwanda (G. Gashaka, unpublished data), Burundi (Bigirimana et al., 2011) and the eastern Democratic Republic of Congo (DRC) (Mulimbi et al., 2012). Although CBSD-like symptoms have been observed in tuberous roots of cassava plants harvested in Bas Congo Province in western DRC (Mahungu et al., 2003), Mulanje Province in central Angola (Lava Kumar et al., 2009) and parts of Madagascar, none of these reports has been verified in spite of extensive diagnostic efforts (Fig. 2a).

Although a definitive cause for the sudden upsurge in CBSD in the Great Lakes region of East and Central Africa is yet to be identified, it seems most likely that this is the result of the dramatic increase in populations of the whitefly vector, B. tabaci, that has occurred in the region since the early 1990s (Legg et al., 2006; Otim-Nape et al., 1996). Up to 100-fold increases in B. tabaci abundance have been recorded in CMD pandemic-affected regions of East and Central Africa, including Uganda, western Kenya, north-western Tanzania, Rwanda, Burundi and eastern DRC (Legg et al., 2006). Evidence has been presented for the association of specific genotypes of B. tabaci with the cassava virus pandemics of East and Central Africa (Legg et al., 2002, 2014b), although it has also been hypothesized that B. tabaci population increases are a consequence of synergistic interactions with CMD-infected cassava host plants (Colvin et al., 2006). Whichever is the case, the upsurge in B. tabaci population is incontrovertible and appears to be the key driver of the new mid-altitude outbreaks of CBSD (Legg et al., 2011, 2014b). These recent changes in the dynamics and distribution of CBSD mean that there is great current concern about the threat of further westward spread within Africa towards Nigeria (Legg et al., 2014a), which is currently the world's largest producer of cassava (FAOSTAT, 2014).

From some of the earliest studies of CBSD, it was noted that the disease causes losses in production through reduced growth as well as spoilage of harvested roots due to necrotic rot (Nichols, 1950). There have been few quantitative assessments of yield losses. The first was conducted in southern coastal Tanzania (Hillocks et al., 2001) and demonstrated that losses of up to 70 % occur in the most susceptible cultivars. It was also noted that root symptoms become increasingly severe as plants mature, and that a secondary effect of CBSD damage is early harvesting by farmers in order to prevent root spoilage. In Malawi, variable effects of CBSD on cassava roots were reported (Gondwe et al., 2002). These included: reduction in the quality of roots caused by pitting, constrictions and root necrosis, as well as effects on the productivity of plants, which included reductions in the number and weight of tuberous roots. The overall estimate of production loss for 2001 was between 20 and 25 %, which was equivalent at the time to a financial loss of US$6–7 million. Efforts have been made to estimate the full economic impact of CBSD in Africa through the development of a framework for the calculation of economic damage (Manyong et al., 2012). This framework highlighted some of the undocumented elements of CBSD yield loss, such as deleterious effects on starch quality of non-necrotic portions of affected roots, as well as the additional labour cost of separating necrotic from non-necrotic portions of affected roots (Jeremiah & Legg, 2008). An overall loss estimate of US$75 million was calculated for all eight countries of East and Central Africa affected (Manyong et al., 2012). It was noted, however, that this was almost certainly a significant underestimate, as several elements of the economic damage framework were not included because there were no data available to allow their estimation. Although some progress has been made, the diverse and complex effects of CBSD on cassava plants and the people who grow, process and consume cassava products are only partially characterized. Substantial additional research on this topic is therefore merited.
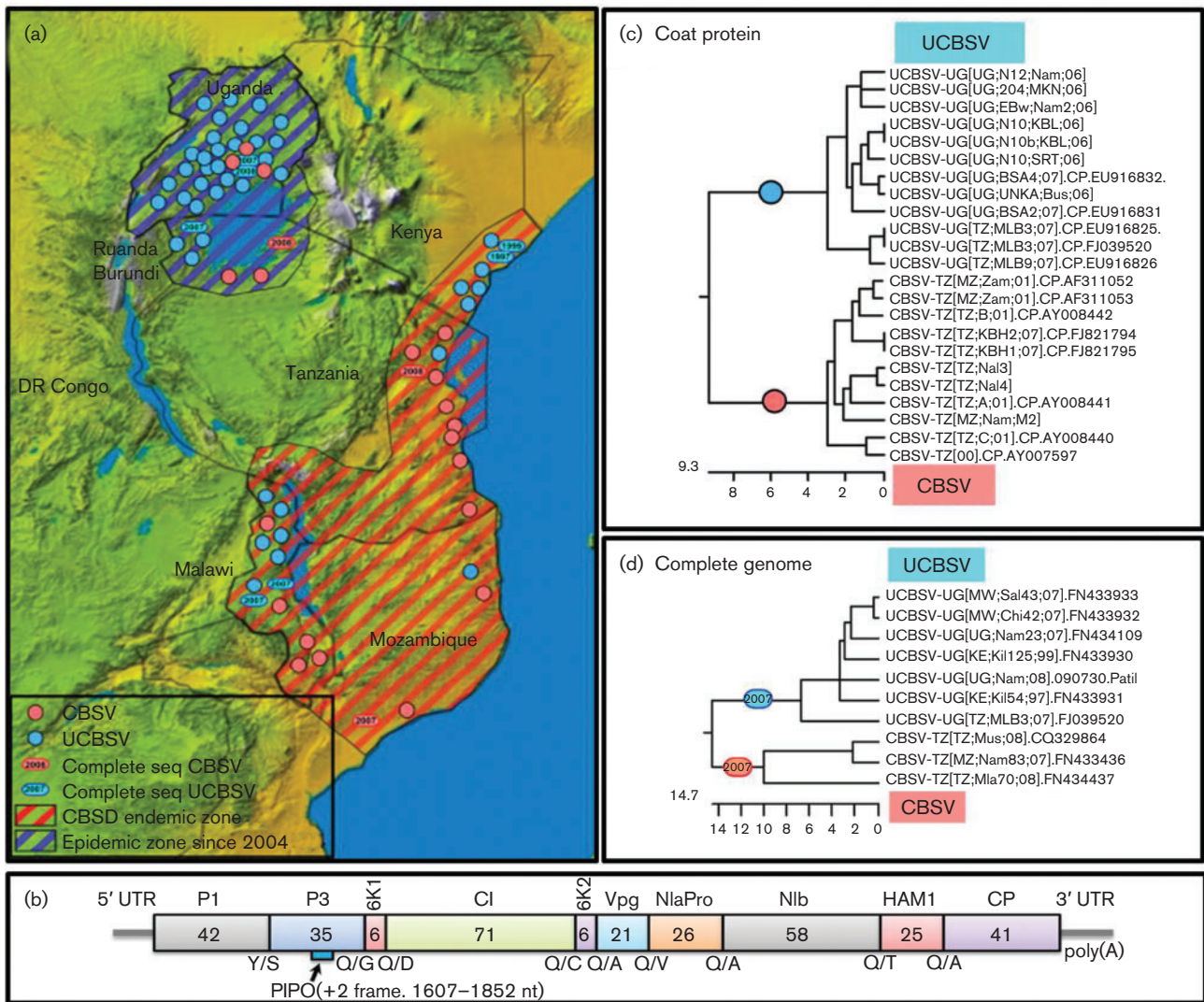
Fig. 2. (a) Spread of CBSD in East Africa shown as endemic (red-striped) and epidemic zones (blue-striped), and distribution of isolates belonging to CBSV (pink circles) and UCBSV (blue circles) in East Africa. Isolates that have been completely sequenced (see d) are indicated in blue and pink ovals, with the year 2007 inscribed in them. (b) Genome organization of UCBSV showing the 10 genes encoded by the 9070 nt genome (P1, first serine proteinase; P3, third protein; 6K1 and 6K2, two 6 kDa proteins; CI, cylindrical inclusion protein; VPg, viral protein genome-linked; NIa-Pro, main viral proteinase; NIb, replicase; Ham1, Maf/HAM1 pyrophosphatase homologue; CP, coat protein) and 3′ and 5′ UTRs, the poly(A) tail and PIPO translated by +2 frame shift within the P3. The amino acids at the predicted proteolytic cleavage sites of the polyprotein are also shown below the polyprotein, and the estimated molecular masses (in kDa) of the mature proteins are indicated in the box for each protein. (c, d) Phylogenetic analysis of CP sequences and complete genome sequences of different isolates of CBSV and UCBSV involved in CBSD. GenBank accession numbers are indicated. UG, Uganda; TZ, Tanzania. Scale bar: pairwise distance expressed as percentage dissimilarity.

Genome organization, genome evolution and gene functions

The viruses causing CBSD belong to the genus *Ipomovirus* of the family *Potyviridae*, with characteristic pinwheel-like or cylindrical inclusions found in the phloem tissue, and with a positive-sense ssRNA genome of ~9 kb (Mbanzibwa *et al.*, 2009a; Monger *et al.*, 2001). The family *Potyviridae* is among the largest of the families of plant viruses, consisting of six genera, distinguished based upon their genomic

organization, sequence relatedness and insect vector (Fauquet, 2007). Viruses of the family *Potyviridae*, except for members of the genus *Bymovirus*, have a monopartite positive-sense ssRNA genome translated into a single polyprotein, which is subsequently auto-cleaved proteolytically by three different proteases into nine to ten individual proteins (Adams *et al.*, 2005). The first complete sequence for a CBSD-associated virus (UCBSV-[TZ:MLB3:07]) was 9069 nt, shorter than the genomes of other ipomoviruses (Mbanzibwa *et al.*,

2009a, b). The 134 nt 5′ UTR is followed by a single long ORF, encoding the polyprotein and then a 3′ UTR of 226 nt (Fig. 2b). For the first CBSV isolates sequenced, the length was ~9008 nt, with a 3′ UTR of 133 nt (Mbanzibwa et al., 2011b; Winter et al., 2010). These UTRs are known to contain regulatory elements for protein translation. The CBSVs are the first members of the family Potyviridae shown to encode a single P1 serine proteinase but lacking HC-Pro (helper component cysteine proteinase) (Fig. 2b) (Mbanzibwa et al., 2009a). The high level of divergence of the P1 protein is characteristic of members of the family Potyviridae (Valli et al., 2007). The P1 of CBSV is most closely related to the P1 of sweet potato mild mottle virus (SPMMV); however, SPMMV contains HC-Pro, indicating it to be an evolutionary link between ipomoviruses and other potyviruses. In contrast, the other two ipomoviruses cucumber vein yellowing virus (CVYV) and squash vein yellowing virus (SqVYV) possess two P1 serine proteinases (P1a and P1b) (Valli et al., 2007). The P1 protein of SPMMV is known to suppress RNA silencing whilst HC-Pro contributes to the durability of silencing suppression (Giner et al., 2008), whereas in the case of ipomoviruses lacking HC-Pro, the P1b protein has taken over the function of silencing suppression (Valli et al., 2006). However, in the case of UCBSV/CBSV, the single P1 protein possesses silencing suppression activity due to the presence of the basic LxKA and Zn-finger motifs (Mbanzibwa et al., 2009a). This LxKA motif in the P1 protein is diverse within different ipomoviruses, with exchanges of lysine (K) and arginine (R) at positions 2 and 3, and in the case of CBSV and UCBSV, the P1 protein contains LRRA (Dombrovsky et al., 2014).

The third protein (P3) and the other seven ORFs (Fig. 2b) encoding two 6 kDa proteins (6K1 and 6K2), cylindrical inclusion protein (CI), viral protein genome-linked (VPg), the main viral proteinase (NIa-Pro), the replicase (NIb) and the coat protein (CP) of CBSV/UCBSV are more similar to the sequences of CVYV, SqVYV and SPMMV (Chung et al., 2008). The P3 protein of CBSV/UCBSV also encodes a second protein, P3N-PIPO, which is generated by a +2 frameshift. It has been demonstrated that the CI and P3N-PIPO complex co-ordinates the formation of plasmodesmata-associated structures that help in the intercellular movement of potyviruses (Wei et al., 2010). In addition to the nine ORFs in the CBSV/UCBSV genome that are characteristic of the family Potyviridae, it also contains an additional ORF (HAM1h) in between the replicase (NIb) and the CP, encoding a polypeptide of 226 aa, flanked by proteolytic cleavage sites. This was unexpected, as the 3′-proximal part of the viral genome is known to be highly conserved among members of the family Potyviridae (Fauquet et al., 2005). This novel sequence had homology with the Maf/HAM1 superfamily of proteins known in many prokaryotes and eukaryotes, which are the nucleoside triphosphate (NTP) pyrophosphatases known to reduce mutations by intercepting non-canonical forms of NTPs and thus preventing their incorporation into nucleic acids, which can lead to unfavourable mutations (Galperin et al.,

2006). The only other virus in which the presence of a HAM1-like sequence has been reported is Euphorbia ringspot virus, which belongs to the genus Potyvirus (Crotty et al., 2001; Fauquet et al., 2005). It might be beneficial to possess an anti-mutator gene under oxidative stress conditions, when the rates of mutations are high. Such conditions could be prevalent in plants of the family Euphorbiaceae, particularly in the older leaves showing early senescence, where CBSV and UCBSV accumulate (Mbanzibwa et al., 2009a). Although there are not many reports on the insertion of heterologous sequences in the plant viral genome, a member of the family Potyviridae, blackberry virus Y, is known to contain AlkB domains within its P1 proteinase, which also counteract deleterious mutations (Susaimuthu et al., 2008). The presence of AlkB domains has also been reported for some viruses in the families Flexiviridae and Closteroviridae (van den Born et al., 2008).

Diversity and distribution of CBSD-causing viruses

Phylogenetic analysis of the CP sequences of virus isolates obtained from CBSD-infected cassava plants from East Africa revealed that these isolates form two distinct clusters (Fig. 2c) (Mbanzibwa et al., 2009a). To date, a total of 12 complete genome sequences of CBSV and UCBSV have been published, which cluster into two distinct phylogenetic groups (Fig. 2d) (Mbanzibwa et al., 2009a, 2011b; Monger et al., 2010; Winter et al., 2010). The complete genomes of CBSV and UCBSV (four and eight isolates, respectively) show an identity of 69.0–70.3 % and 73.6–74.4 % at the nucleotide and polyprotein amino acid sequence levels, respectively. In view of the sequence divergence between these two groups, two distinct species have been recognized, CBSV and UCBSV (Adams & Carstens, 2012; Mbanzibwa et al., 2009b, 2011b; Winter et al., 2010) (Fig. 2d). Under field conditions, there are no diagnostic symptom differences between CBSV and UCBSV infections, although the environmental conditions and the cassava genotype affect symptom severity. However, under controlled conditions there are distinct phenotypes produced by some CBSV and UCBSV isolates, as discussed previously (Mbanzibwa et al., 2011b; Mohammed et al., 2012; Winter et al., 2010). Mixed infections of CBSV and UCBSV occur frequently in areas where the two virus species occur (Mbanzibwa et al., 2011a), although there is no evidence that they interact synergistically, as is the case with African cassava mosaic virus and East African cassava mosaic virus.

Nucleotide identities ranged from ~87–99 % among UCBSV isolates and ~79–95 % among CBSV isolates but are only ~70 % between UCBSV and CBSV isolates (Mbanzibwa et al., 2011b). Analysis of complete genome sequences of CBSV and UCBSV isolates predicted that there were at least two recombination points within the isolates of either CBSV or UCBSV, located at the 3′ end of the genome within the HAM1h- and CP-encoding sequences and in the 3′ UTR (Mbanzibwa et al., 2011b). However, no such recombinations

were detected between isolates of CBSV and UCBSV (Mbanzibwa *et al.*, 2011b), a pattern that is consistent with previous reports on the absence of recombination between diverse genomes of RNA viruses (Chare & Holmes, 2006), possibly because of reduced fitness in recombinants. There were size differences in the sequences encoding the CI, VPg and CP proteins for the isolates of each species. The HAM1h sequences of these two species displayed the lowest amino acid identity (<55 %), indicating that these two viruses either acquired HAM1h from two different hosts at two different time points after speciation or that HAM1h evolved more rapidly than the other genes, which is also evidenced by the adaptive selection pressure on HAM1h for both CBSV and UCBSV. Questions about the origin of HAM1h sequences may be resolved as further progress is made in sequencing plant genomes and in particular the cassava genome (Prochnik *et al.*, 2012). However, there were 33 highly conserved amino acid residues across HAM1 homologues, present in different organisms, and with indications of a strong negative selection on HAM1h and CP genes (Mbanzibwa *et al.*, 2009a, 2011b). The size of the CBSV CP was 9 aa longer than the CP of UCBSV, and the sizes of CBSV/UCBSV P1 proteins were divergent, with only ~60 % amino acid identity between isolates of the two species (Mbanzibwa *et al.*, 2011b; Winter *et al.*, 2010). The size difference in CP sequences corresponds to the different genome sizes of CBSV and UCBSV and might also contribute to differential transmission by the whitefly vector, although there is currently no evidence for this. The diversity in the sequences of P1 may contribute to variation in their ability to suppress RNA silencing and thus a marked difference in the virulence of CBSV and UCBSV isolates.

Phytosanitary practices to manage CBSD

Like other virus diseases of vegetatively propagated crops, phytosanitary practices can play a major role in limiting the impact and spread of CBSD (Hillocks & Jennings, 2003; Storey, 1936). In view of the cryptic symptoms of CBSD, where symptoms are typically mild and mainly confined to lower leaves, it can be difficult to distinguish between healthy and infected plants. This has the consequence that CBSVs are readily propagated through infected planting material. Additionally, the semi-persistent transmission of these viruses means that they are retained for relatively short periods of time, limiting the distance over which they can be carried by their whitefly vector (Jeremiah, 2014). CBSD therefore appears to be spread by vectors over relatively short distance but is readily carried longer distances through transport of planting material. This contrasts with the CMGs causing CMD, which can be carried over long distances by whiteflies but are less likely to be propagated through planting material as their symptoms are much more obvious (Legg *et al.*, 2011). In view of these biological characteristics, phytosanitation is of much greater importance for CBSD than it is for CMD. Major components of CBSD control programmes, therefore, include:

1. The production of 'clean' stocks of planting material, including virus indexing of parent material in tissue culture, systematic virus testing in isolated pre-basic germplasm multiplication and regular roguing of symptomatic plants in the propagation field.

2. Collective action at a community level to encourage groups of farmers growing cassava in close proximity to one another to co-operate in implementing phytosanitary measures, including the sourcing of 'clean' planting material and its maintenance through roguing and selection of healthy stems for replanting.

Large-scale initiatives are currently being implemented in parts of eastern and southern Africa and are using these approaches to constrain both local and regional spread of CBSD. In addition, the importance has been emphasized for national and subregional-level quarantine authorities to enforce effective controls on intra- and inter-continental movements of cassava germplasm in order to ensure that CBSD does not spread beyond its currently confined distribution in eastern and southern Africa (Legg *et al.*, 2014b).

Breeding for CBSD resistance and sources of resistance

The most effective and realistic approach in reducing losses due to diseases is the use of host-plant resistance or the deployment of less-susceptible cultivars. Breeding in cassava is a major challenge, as it is cross-pollinated and highly heterozygous (Ceballos *et al.*, 2012). Breeding for resistance to CMD and CBSD started in 1935 at Amani, Tanzania (Hahn *et al.*, 1980; Jennings, 1960). Failure to identify good sources of CMD resistance from a worldwide collection of cassava cultivars led to the lengthy process of transferring genes for resistance to both CMD and CBSD from related species (Jennings, 2003; Nichols, 1947). The most resistant variety developed from this programme was 46106/27, which was a third back-cross derivative from *M. esculenta* × *M. glaziovii* (Jennings, 2003; Nichols, 1947). It is probably the most successful product of the Amani research programme that is presently available to farmers and whose resistance to CBSD has persisted for many years in farmers' fields in Kenya, where it is locally known as Kaleso (Hillocks & Jennings, 2003). More than 500 single-nucleotide polymorphism (SNP) markers have recently been used to show that Kaleso is genetically identical to the cultivar Namikonga, which is grown in Tanzania and is considered to be the best source of CBSD resistance (Pariyo *et al.*, 2013). These SNP markers have been placed on an integrated SNP-simple sequence repeat genetic linkage map, which are used for quantitative trait locus (QTL) detection of tolerance to CBSD (Ferguson *et al.*, 2012; Kulembeka *et al.*, 2012; Pariyo *et al.*, 2013; Rabbi *et al.*, 2012). QTLs associated with CBSD resistance were also identified by generating a mapping population of 60 F1s from a cross between the CBSD-tolerant cultivar, Namikonga and a susceptible cultivar, Albert (Morag Ferguson, personal

communication). The availability of the cassava genome sequence (Prochnik et al., 2012; Wang et al., 2014) should help in identifying genes controlling CBSD resistance, as well as novel markers associated with CBSD resistance. Efforts are on going to identify CBSD resistance genes by RNA-sequencing and transcriptome profiling of CBSD-resistant and -susceptible cassava cultivars. RNA sequencing is a technology that uses the capabilities of next-generation sequencing for whole-transcriptome shotgun sequencing to study gene expression at a given moment of time. Recently three varieties of cassava – Kaleso (highly resistant to CBSD), Kiroba (moderately resistant to CBSD) and Albert (highly susceptible to CBSD) – were challenged with CBSD and then subjected to Illumina RNA sequencing (Maruthi et al., 2014). Sequence analysis showed over-expression of more than 700 genes in CBSD-resistant Kaleso in comparison with Albert. Although virtually none of the overexpressed genes resembled known resistance gene ortho-logues, some genes encoded enzymes or factors involved in hormone signalling pathways and secondary metabolites, both of which are linked to disease resistance (Maruthi et al., 2014).

Several CBSD-resistant clones have been identified in Kenya (Kaleso, Guzo, Gushe, Kibiriti Mweusi and Ambari), Mozambique (Nikwaha, Chigoma Mafia, Nanchinyaya, Xino Nn'goe, Likonde, Mulaleia and Badge) and Tanzania (Namikonga, Kiroba, Nanchinyaya, Kigoma Mafia, Kitumbua, Kalulu, Mfaransa, Muzege, Gezaulole and Kibangameno). Some of these clones are former Amani hybrids that are no longer recognized as such, as they have been given local names. Most of these are better described as 'tolerant', as they readily show foliar symptoms but root necrosis is delayed or absent (Hillocks & Jennings, 2003) (Fig. 3).

The exchange of virus-resistant cassava germplasm is one of the principle activities of the International Institute of Tropical Agriculture-led project 'New Cassava Varieties and Clean Seed to Combat CMD and CBSD' funded by the Bill and Melinda Gates Foundation, which was initiated in 2012 and will run through to 2016. The project aims to ensure that farmers have access to diverse disease-free improved varieties with combined resistance to CBSD and CMD, as well as preferred end-user characteristics. These are now being used extensively in breeding programmes as sources of resistance to generate new improved clones. Inter-crossing among these will concentrate resistance genes and allow recessive genes to be expressed (Hillocks & Jennings, 2003). Some of the F1 progeny remain symptom free after being challenged with the virus or show a low incidence of infection and reduced symptom severity. Both additive and non-additive genetic effects (Holland, 2001) have recently been reported to be important in the expression of CBSD resistance, and in studies of these effects, Kaleso (Namikonga) had the highest general combining ability for resistance to CBSD (Kulembeka et al., 2012; Mtunda, 2009; Munga, 2008).

Genetic engineering for CBSD resistance

There are several strategies available for controlling plant viruses by genetic engineering, which have been reviewed extensively by Sudarshana et al. (2007) and Reddy et al. (2009). Although natural sources of resistance for CBSD are available, which can be introgressed into farmer-preferred cassava cultivars through conventional breeding, in practice it is difficult to combine CBSD resistance with good root and harvest qualities (Jennings, 2003). In view of the difficulties associated with conventional breeding, genetic engineering offers great potential for the rapid transfer of resistance genes to traditional cultivars, bypassing the possibility of the appearance of undesirable traits. Among the available biotechnological approaches, RNA interference (RNAi) or gene silencing technology, also called post-transcriptional gene silencing, offers significant potential for the control of CBSD (Patil et al., 2011; Prins et al., 2008; Reddy et al., 2009). In collaboration with different African institutes, at least three laboratories, located in the USA (Danforth Plant Science Center, St Louis), Switzerland (ETH, Zurich) and Germany (DSMZ Plant Virus Department, Braunschweig) are working on developing CBSD-resistant transgenic cassava by employing RNAi technology. In addition to the hairpin RNA constructs, artificial microRNA constructs targeting different regions of CBSV/UCBSV have been developed and significant resistance was obtained in transgenic N. benthamiana (Niu et al., 2006; Wagaba H., Patil B. L., Mukasa S., Baguma Y., Alicai T., Fauquet C. M., Taylor N. J., unpublished data).

Through a collaborative project called Virus Resistant Cassava for Africa (VIRCA), researchers at the Danforth Plant Science Center with two partner institutions in Africa, the National Crops Resources Research Institute (NaCRRI, Namulonge) in Uganda and the Kenya Agricultural Research Institute (KARI, Nairobi) have demonstrated the proof of concept for the control of CBSD by RNAi (Ezezika et al., 2012; Taylor et al., 2012). Three RNAi constructs targeting different parts of the CP of UCBSV-[UG:Nam:04] were generated, which consisted of the 894 nt (FL, full-length), 397 nt N-terminal (NT) and 491 nt C-terminal (CT) portions of the CP and expressed constitutively in the model host N. benthamiana (Fig. 4a) (Patil et al., 2011). In inoculation studies with UCBSV-[UG:Nam:04], plants homozygous for FL-CP showed the highest resistance (100 % resistance for 85 % of the plant lines screened), followed by the NT and CT parts of CP, which also provided 100 % resistance in some of the plant lines (Fig. 4a). Further cross-protection studies with non-homologous CBSV isolates demonstrated that some of the lines derived from FL-CP showed 100 % protection. These results comprised the first demonstration of RNAi-mediated cross-protection to members of two different species with more than 25 % sequence dissimilarity (Patil et al., 2011). The level of virus resistance obtained in different transgenic plant lines had a positive correlation with the level of siRNA expression and also with expression of the transgene. Transgenic cassava (cultivar TMS60444) plants were also generated by transforming these RNAi constructs and, when graft challenged with UCBSV-infected scions, some of the transgenic lines
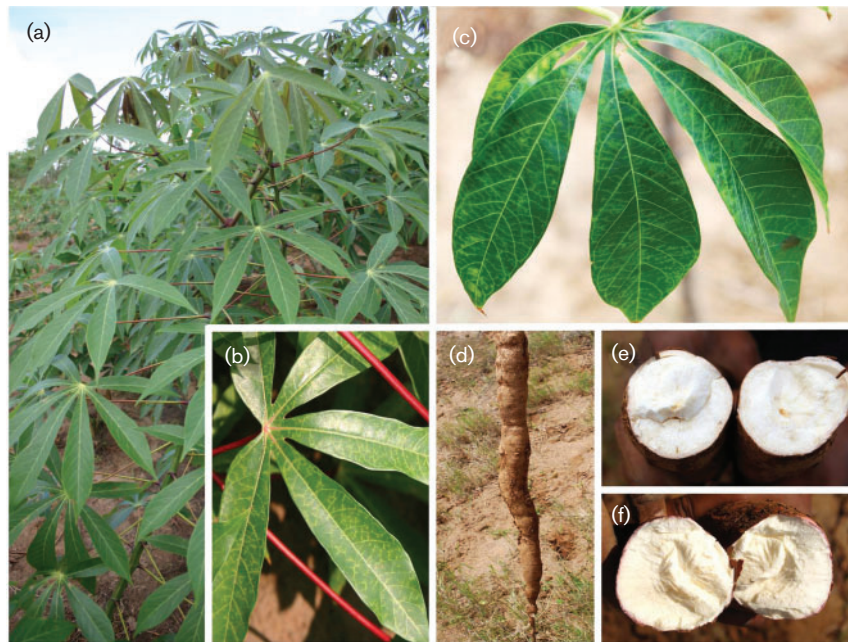
Fig. 3. Symptoms of CBSD-tolerant cassava cultivars. (a) Very mild symptoms on leaves of cv. NDL 2006/132. (b) Close-up of CBSD symptoms on leaves of cv. NDL 2006/132. (c) Mild vein clearing symptoms on leaves of cv. Kiroba. (d) Slight root constriction on roots of cv. Namikonga. (e) Extremely mild necrotic symptoms in roots of cv. Namikonga. (f) Absence of necrosis in roots of the cv. Kiroba.

were immune to infection by this homologous virus (Yadav *et al.*, 2011). The seven best transgenic RNAi lines derived from each gene construct of FL-CP (pILTAB 718) and NT-CP (pILTAB 719) were evaluated under confined field trials at NaCRRI, at Namulonge in Uganda (Fig. 4b) (Ogwok *et al.*, 2012). All the transgenic lines derived from FL-CP showed significant tolerance to CBSD, whilst 90 % of the non-transgenic control plants were heavily infected and later developed severe root necrosis. Of all the transgenic lines, the line 718-001 showed near immunity to both UCBSV and CBSV, which was free of both foliar and root necrotic symptoms during the entire period of the field trial (Fig. 4 c–f). Most of the non-transgenic control plants were infected with UCBSV; however, the few transgenic plants that showed CBSD symptoms were infected with the non-homologous virus CBSV, whereas the transgenic line 718-001, expressing very high levels of small interfering RNA (siRNA) (Fig. 4 g), was free from both UCBSV and CBSV (Ogwok *et al.*, 2012). This cross-protection against the non-homologous CBSV by siRNAs generated from the UCBSV FL-CP confirms the initial observations made in transgenic *N. benthamiana* (Patil *et al.*, 2011). The performance of these resistant transgenic cassava lines has also been evaluated for the durability of resistance and siRNA expression in subsequent generations, by further propagation of their stem cuttings (Odipio *et al.*, 2014).

The best RNAi constructs for CBSV and UCBSV are now being transformed into farmer-preferred cultivars of cassava

with the objective of producing virus-resistant cassava for African farmers. However, other regions of the CBSV/UCBSV genome outside the CP need to be evaluated for their potential to control CBSD through RNAi. It should also be possible to combine transgenes for CBSVs with others identified for resistance to CMGs and thereby achieve resistance to multiple viruses infecting cassava. Recently, Vanderschuren *et al.* (2012) generated transgenic cassava lines by transforming the CMD-resistant Nigerian landrace TME7 (Oko-Iyawo) with the CBSV CP RNAi construct. The main hurdles in screening transgenic cassava lines for CBSD resistance have been the lack of infectious clones of UCBSV or CBSV, and poor whitefly transmission under greenhouse conditions (Maruthi *et al.*, 2005). Consequently, labour-intensive grafting is the only option for screening CBSD resistance prior to field trials.

RNAi-mediated field resistance has also been demonstrated for papaya (Fuchs & Gonsalves, 2007), squash (Tricoli *et al.*, 1995) and plum (Hily *et al.*, 2004), which are approved and commercially cultivated by farmers. However, the major concern in using RNAi technology to control plant viruses is that point mutations or recombinations in the target virus could 'break' the engineered resistance. Thus, different strategies will need to be developed to counter this potential risk of resistance breakdown. However, despite these drawbacks, genetic engineering continues to offer great promise as an effective strategy to control these devastating viruses.
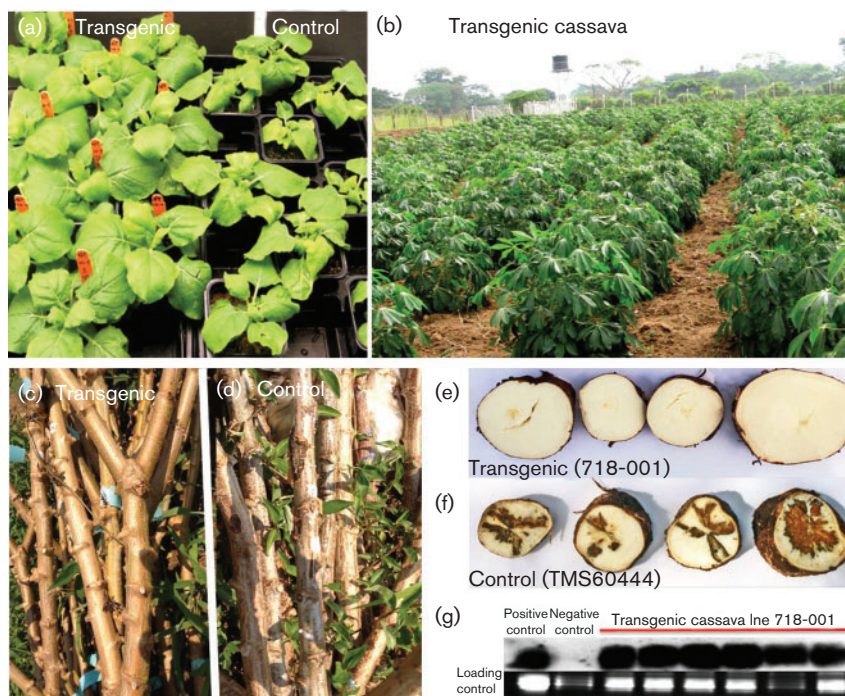
Fig. 4. (a) Screening of RNAi-transgenic *N. benthamiana* lines in the T2 generation for resistance against UCBSV (Patil *et al.*, 2011). (b) Field resistance to CBSD in RNAi-based transgenic cassava lines in the confined field trials conducted at the Namulonge research station in Uganda (Odipio *et al.*, 2014; Ogwok *et al.*, 2012). (c, d) Dark brown necrotic lesions seen on stems of CBSD-infected plants (d) compared with non-infected cassava plants (c). (e, f) CBSD symptom distribution in roots from harvested transgenic (e) and non-transgenic (f) cassava plants. (g) Small interfering RNA accumulation in transgenic cassava for the full-length (FL)-ΔCP (pILTAB718) by Northern blotting (Ogwok *et al.*, 2012). The negative control is RNA from a healthy non-transgenic (TMS60444) plant and the positive control is the original transgenic plant (718-001) used for propagation in the field trials.

Conclusions and future prospects

There are major differences in the regional epidemiology of the CMD and CBSD pandemics, and thus specific disease management strategies need to be designed for each of these two important viral diseases of cassava (Legg *et al.*, 2011). Although the control of whitefly is most critical to prevent the dissemination of cassava viruses, the role of vectors is slightly less important for CBSVs than it is for CMGs, as CBSVs are transmitted semi-persistently. Hence, farmers play a more significant role in the dissemination of CBSD through infected cuttings, thus emphasizing the importance of the distribution of healthy planting material to restrict the spread of CBSD. It is very important to establish and enforce strict quarantine on the exchange of cassava germplasm between countries, regions and continents in order to prevent accidental introduction of CBSD to countries where it is currently absent. Overall, for successful management of CBSD, major emphasis needs to be placed on phytosanitary measures, mandatory virus indexing for germplasm exchange and surveillance of CBSD epidemics.

CBSD was first reported in 1935, but prior to the end of the 20th century, studies on CBSD were restricted to the field

and barely any molecular characterization had been attempted. Only after the recent epidemic was first noted in 2004 has the disease been subject to greater molecular scrutiny. Much still remains to be done, however, in order to generate more comprehensive molecular and biological information for these viruses. The genome organization of CBSV is unique within members of the family *Potyviridae*, and novel proteins like HAM1h need thorough characterization. In addition, much more detailed data are required on virus–vector interactions and transmission. Although several whole genomes have now been sequenced and are publicly available, there are many gaps in sequence information from more recently affected countries, such as Rwanda, Burundi and DRC. Additional data of this type will strengthen our understanding of the probable origin and patterns of evolution of the CBSVs. Next-generation sequencing technologies can have an important future role in identifying the causative agent(s) of CBSD-like symptoms in plants that test negative for CBSVs with existing diagnostics, as well as in identifying possible alternative hosts. Co-ordinated cassava breeding programmes need to be encouraged that will deploy novel and robust molecular markers, deduced from the sequence of the cassava

genome. Identification, isolation and characterization of resistance genes from wild relatives of cassava will greatly help in gene pyramiding and in achieving broad-spectrum resistance. The successful field demonstration of resistance to CBSD using RNAi technology is a major milestone in addressing the concerns of CBSD control. However, accelerated field testing of transgenic plants developed in farmers' preferred cultivars, resistant to both viruses (CBSV and UCBSV) as well as to the most prevalent CMGs, should be an important priority. In addition, other virus resistance technologies should be evaluated in order to provide stronger and more durable resistance.

Although there are growing investments being made by donor agencies for research efforts on CBSD, the scale of these investments and the extent of research and development co-ordination need to be improved to respond adequately to the urgent needs of farmers in Africa.

Acknowledgements

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