Leke et al. Agriculture & Food Security (2015) 4:1
DOI 10.1186/s40066-014-0020-2

Agriculture & Food Security

REVIEW Open Access

Begomovirus disease complex: emerging threat to vegetable production systems of West and Central Africa

Walter N Leke[1,2*], Djana B Mignouna[1], Judith K Brown[3] and Anders Kvarnheden[4]

Abstract

Vegetables play a major role in the livelihoods of the rural poor in Africa. Among major constraints to vegetable production worldwide are diseases caused by a group of viruses belonging to the genus *Begomovirus*, family *Geminiviridae*. Begomoviruses are plant-infecting viruses, which are transmitted by the whitefly vector *Bemisia tabaci* and have been known to cause extreme yield reduction in a number of economically important vegetables around the world. Several begomoviruses have been detected infecting vegetable crops in West and Central Africa (WCA). Small single stranded circular molecules, alphasatellites and betasatellites, which are about half the size of their helper begomovirus genome, have also been detected in plants infected by begomoviruses. In WCA, *B. tabaci* has been associated with suspected begomovirus infections in many vegetable crops and weed species. Sequencing of viral genomes from crops such as okra resulted in the identification of two previously known begomovirus species (*Cotton leaf curl Gezira virus* and *Okra yellow crinkle virus*) as well as a new recombinant begomovirus species (*Okra leaf curl Cameroon virus*), a betasatellite (*Cotton leaf curl Gezira betasatellite*) and new alphasatellites. Tomato and pepper plants with leaf curling were shown to contain isolates of new begomoviruses, collectively referred to as West African tomato-infecting begomoviruses (WATIBs), new alphasatellites and betasatellites. To study the potential of weeds serving as begomovirus reservoirs, begomoviruses and satellites in the weed *Ageratum conyzoides* were characterized. Sequence analyses showed that they were infected by isolates of a new begomovirus (*Ageratum leaf curl Cameroon virus*) that belong to the WATIBs group, a new betasatellite (*Ageratum leaf curl Cameroon betasatellite*), an alphasatellite and two types of defective recombinants between a begomovirus and an alphasatellite. Putative recombinations were detected in begomovirus genomes for all four plant species studied, indicating that recombination is an important mechanism for their evolution. A close relationship between the begomoviruses infecting pepper and tomato and *A. conyzoides* and the detection of the same alphasatellite in them support the idea that weeds are important reservoirs for begomoviruses and their satellites. With this high diversity, recombination potential and transmission by *B. tabaci*, begomoviruses and ssDNA satellites pose a serious threat to crop production in West and Central Africa.

Keywords: Begomoviruses, Okra leaf curl disease, Whitefly, Tomato leaf curl disease, West and Central Africa, Viral satellites

* Correspondence: wnleke@yahoo.com
[1]International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo road, Ibadan, Nigeria
[2]Institute of Agricultural Research for Development (IRAD), P.O. Box 2123, Messa, Yaoundé, Cameroon
Full list of author information is available at the end of the article

Introduction

The population size of West and Central Africa (WCA) is approximately 350 million people with about 80% depending on agriculture for their livelihoods. Vegetables play an essential role in the diet, health, and livelihoods of the rural poor in WCA. A survey conducted by the Natural Resources Institute in Cameroon and Uganda provides evidence that vegetables offer a significant opportunity for poor people to earn a leaving [1]. Among the major constraints to vegetable crop production is a group of viruses belonging to the family *Geminiviridae*. Geminiviruses are arguably the most damaging plant viruses worldwide and pose a severe threat to global food security. They are abundant in tropical and subtropical environments, where insects that transmit these viruses are abundant. Although geminiviruses have been identified as plant pathogens for many years [2,3], the advent of modern cropping practices has made these viruses more widespread, particularly to monoculture vegetable crop farms [4,5]. Geminiviruses are divided into seven genera—*Becurtovirus*, *Begomovirus, Eragrovirus*, *Mastrevirus, Curtovirus, Topocuvirus*, and *Turncurtovirus*—based on genome organization, nucleotide sequence similarities, and biological properties [6]. The genome of viruses in the genus *Begomovirus* consists of either two genomic components, bipartite (known as DNA-A and DNA-B) of about equal size (~2.8 kb; example *East African cassava mosaic Cameroon virus*, EACMCV) (Figure 1A) or a single component, monopartite, homologous to the DNA-A component of bipartite viruses (example *Tomato leaf curl Cameroon virus*, ToLCCMV) (Figure 1B) [7,8]. Begomoviruses cause many diseases of dicotyledonous crops and wild plants. The symptoms typically consist of leaf curling, mosaic, vein yellowing, or more generalized leaf yellowing, often accompanied by stunting of plant growth (Figure 2). Some of these diseases are among the world's most economically important plant virus diseases, for example, mosaic diseases of cassava in

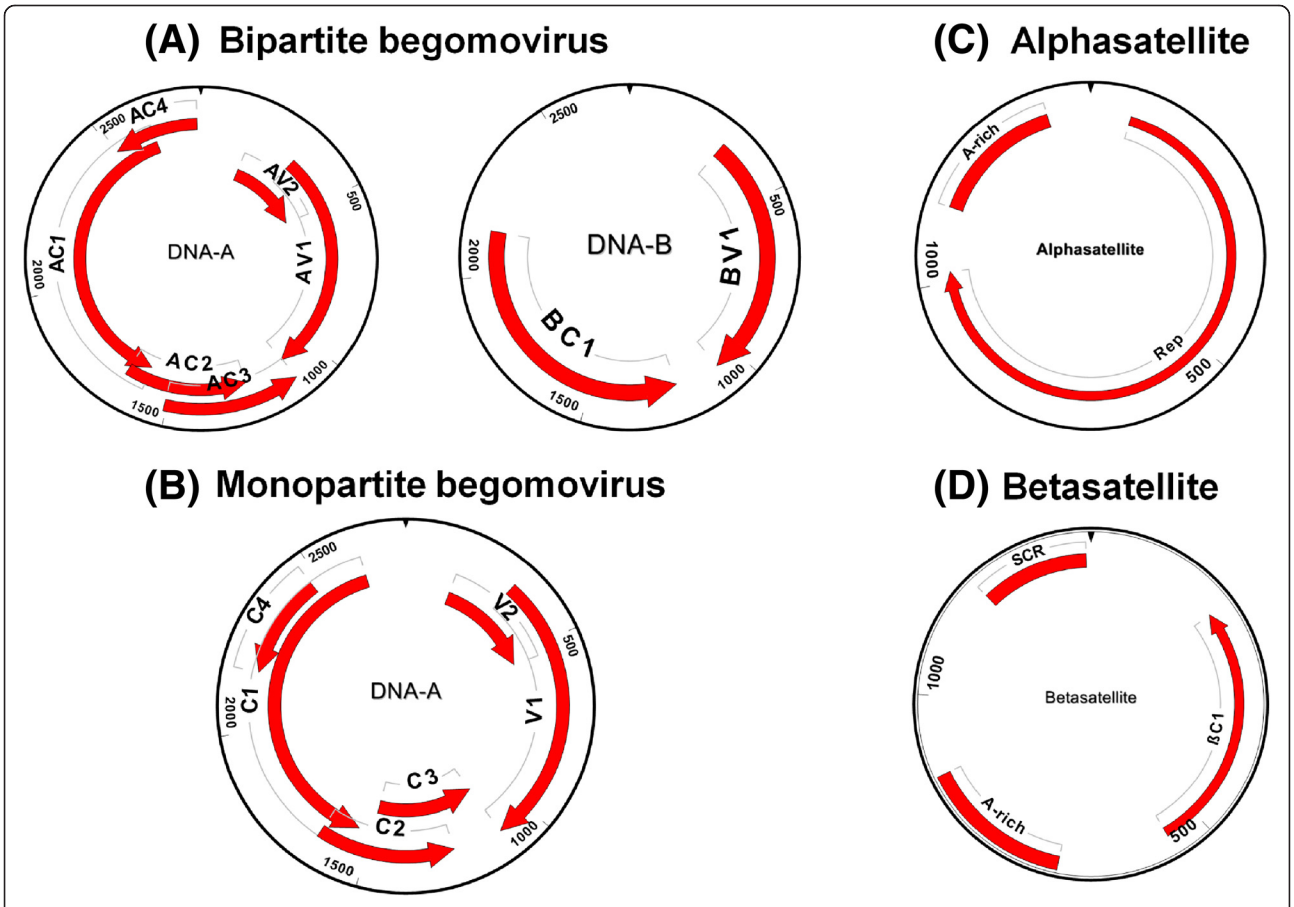

Figure 1 Genome organization of the bipartite (A) and monopartite (B) members of the genus Begomovirus, alphasatellites (C) and betasatellites (D). *Arrows* represent open reading frames. *V1*/(*AV1*) coat protein gene, *V2*/*AV2* precoat prorein gene, *C1* (*AC1*) replication initiator protein gene, *C2* (*AC2*) transcription activator protein gene, *C3* (*AC3*) replication enhancer protein gene, *C4* (*AC4*) symptom determinant protein gene, *BV1* nuclear shuttle protein gene, and *BC1* movement protein gene. A-rich: adenosine-rich region of the genome; *βC1*: the gene encoding the betaC1 protein; SCR: satellite-conserved region. Imagines are not to the scale.

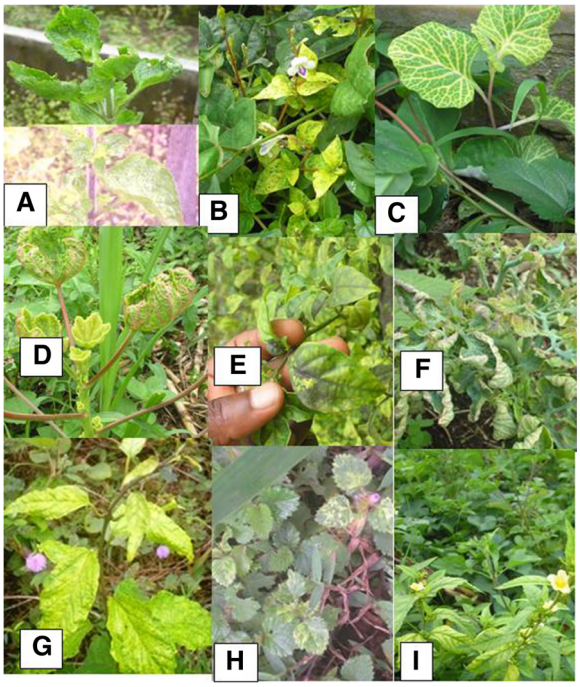

Figure 2 Symptoms in begomovirus infected crops and weeds in West and Central Africa. (A) *Ageratum conyzoides*; **(B)** *Asystasia gangetica*; **(C)** *Emilia cocinea*; **(D)** Okra, *Abelmoschus esculentus*; **(E)** Pepper, *Capsicum annuum*; **(F)** Tomato, *Solanum lycopersicum*; **(G)** *Clerodendrum umbellatum*; **(H)** *Malvastrum* spp.; and **(I)** *Sida corymbosa.*

Sub-Saharan Africa probably cause annual yield losses exceeding $2 billion in value of a staple food for millions of poor people [9].

Genome organization of bipartite begomoviruses

Except for a short sequence of ~200 nts, referred to as the 'common region' (CR), the DNA-A and DNA-B components share no sequence similarity. The CR contains the nonanucleotide TAATATTAC sequence, where rolling circle replication is initiated, and that is conserved among members of the family *Geminiviridae* [10-12]. Both genomic components contain protein-coding sequences on the viral sense strand and on the complementary strand. Six genes seem to be universally present in all bipartite begomoviruses. The DNA-A component contains one gene (*AV1*) on the viral sense strand and three genes (*AC1*, *AC2*, *AC3*) on the complementary strand for the New World (NW) bipartite begomoviruses [13] and an additional gene *AV2* in the viral sense strand and *C4* on the complementary strand for the Old World (OW) bipartite begomoviruses [14]. The sense and complementary strands of the DNA-B component each contains one gene, *BV1* and *BC1*, respectively [15] (Figure 1A).

Genome organization of monopartite begomoviruses

The genome of monopartite begomoviruses contains six open reading frames (ORFs). The coat protein gene (*CP* or *V1*), and *V2,* are expressed from the viral sense strand, and *C1* (replication-associated (activator) protein (Rep)), *C2*, *C3*, and *C4*, are expressed from the complementary strand [16] (Figure 1B).

DNA satellite molecules associated with monopartite begomoviruses

Satellites are defined as viruses or nucleic acids that depend on the helper virus for their replication but lack extensive nucleotide sequence identity to the helper virus and are dispensable for its proliferation [17]. Satellite viruses encode a structural protein, which encapsidates its own nucleic acid, while satellite nucleic acids rely on the helper virus structural protein for encapsidation and do not necessarily encode additional non-structural proteins. A third type of agent, referred to as satellite-like nucleic acid, also depends on the helper virus for its replication but provides a function that is necessary for the biological success of the helper virus and is therefore considered as part of the helper virus genome [18]. The first satellite RNA was identified in 1969 in association with the nepovirus *Tobacco ringspot virus* [18], and since then, a large number of satellite RNAs, associated with several groups of plant viruses, have been reported [17], with the majority of the satellites interfering with the replication of the helper viruses, resulting in attenuated symptoms. Some satellites exacerbate disease symptoms induced by the helper virus or produce novel symptoms which are usually not associated with the helper virus infections [19].

The first begomovirus satellite DNA to be discovered was found to be associated with the monopartite begomovirus *Tomato leaf curl virus* (ToLCV) from Australia [20]. This 682 nt circular ssDNA depends on ToLCV for its replication and encapsidation, but its replication can also be supported by other begomoviruses. It has no proven effects on the viral replication or on symptoms caused by ToLCV. It has no extensive ORFs and has little sequence similarity to its helper virus (ToLCV) except for the nonanucleotide TAATATTAC sequence presents in the stem loop of all geminiviruses [21]. Failure to reproduce yellow vein symptoms in *Ageratum conyzoides* (goat weed) by re-introduction of *Ageratum yellow vein virus* (AYVV) [22,23], suggested that another factor was required to restore pathogenicity in the natural host. In a search for additional viral components, a number of small circular recombinant components, each containing the AYVV origin of replication together with sequences of unknown origin, were isolated from infected goat weed [24]. Similar recombinants were also identified for the begomoviruses associated with cotton

leaf curl disease (CLCuD) [25-27]. The significance of the unidentified sequences within the recombinants was not appreciated at the time, but they were to provide a vital clue in the discovery of a new class of satellites. As was observed for AYVV, the cloned genomic component of cotton leaf curl virus (renamed *Cotton leaf curl Multan virus*, CLCuMV) failed to induce typical CLCuD symptoms, suggesting the presence of another factor [26], whose search resulted in the isolation of a small circular ssDNA molecule, referred to as DNA-1 [28], which is a representative of a new class of components associated with monopartite begomoviruses [29].

Recently, many monopartite begomoviruses have been identified that associate with a type of satellite molecule referred to as betasatellite, composed of ssDNA, ~1.3 kb in size and approximately half the size of the helper begomoviruses (Figure 1D). Many of the betasatellites are required for typical disease symptom development [22,27,30-33]. Despite their recent discovery, betasatellites may have existed for many centuries, e.g., Eupatorium yellow vein betasatellite.

(EpYVB) in association with *Eupatorium yellow vein virus* (EpYVV) have been demonstrated to cause eupatorium yellow vein disease (EpYVD), which was described about 1250 years ago [2]. All betasatellites require a helper begomovirus for replication, local and systemic spread, and whitefly vector-mediated transmission, and some have been shown to modulate symptom severity [33]. To further show the role that betasatellites play in begomoviral disease etiology, the β*C1* (Figure 1D) has in one instance been shown to be responsible for the suppression of jasmonic acid signaling involved in at least one gene silencing pathway [34]. The transgenic plants of *Arabidopsis thaliana* expressing β*C1* of *Tomato yellow leaf curl China betasatellite* (TYLCCNB) were shown to develop disease symptoms like that observed in begomovirus-infected tobacco plants, in that plants exhibited upward leaf curling, foliar enations, and sterile flowers [35]. All betasatellite molecules contain one ORF (β*C1*) (Figure 1D), an A-rich region ~240 nts long and a satellite-conserved region (SCR), of ~220 nts. Apart from the nonanucleotide sequence, betasatellites do not share any significant sequence similarity with the helper begomoviruses.

The begomovirus/betasatellite complexes are often associated with a second type of circular ssDNA satellite, initially referred to as DNA-1 [28,29,36,37], but now called alphasatellites [38]. Alphasatellites encode a single protein that shares high nt identity with the Rep (Figure 1C), a rolling-circle replication initiator protein encoded by viruses in the genus *Nanovirus*, family *Nanoviridae* that also have a genome of circular ssDNA [39]. Consequently, alphasatellites are capable of autonomous replication but require a helper begomovirus for spread in plants and for whitefly vector transmission. In addition to Rep, alphasatellites also have an A-rich region, ~200 nts long, downstream of the Rep-encoding region. Recently, it has been demonstrated that the alphasatellite associated with *Tobacco curly shoot virus* (TbCSV) can be used as a virus-induced gene silencing (VIGS) vector [40]. In contrast to betasatellites, alphasatellites possess in their stem loop the nonanucleotide sequence TAGTATTAC which is also found in the stem loop of viruses in the family *Nanoviridae*. Alphasatellites can affect both begomovirus titer and symptom development in host plants [36,41]. Initially it was thought that satellite molecules were limited to the OW, but recently, alphasatellites have been found associated with NW begomoviruses [42,43], thus expanding the geographical distribution of satellite molecules associated with begomoviruses.

Diversity and distribution of begomoviruses and ssDNA satellites in West and Central Africa

Until recently, knowledge on the prevalence and impact of begomoviruses in West and Central Africa has been rather scanty. Initially, information on the existence of begomoviruses infecting crops in the region was based on serology and hybridization, with emphasis on cassava mosaic disease (CMD), okra leaf curl disease (OLCD) and tomato leaf curl disease (ToLCD) [44-48]. With the advent of more advanced molecular techniques in the study of begomoviruses, such as polymerase chain reaction (PCR), rolling cycle amplification (RCA)/restriction fragment length polymorphism (RFLP), and sequencing, the situation has greatly improved, leading to the identification of previously unknown begomovirus/satellite complexes infecting tomato, okra and pepper such as *Tomato leaf curl Cameroon virus* (ToLCCMV), *Tomato leaf curl Nigeria virus* (ToLCNGV), *Tomato leaf curl Ghana virus* (ToLCGHV), *Tomato leaf curl Kumasi virus* (ToLCKuV), and *Tomato leaf curl Togo virus* (ToLCTGV), which could collectively be termed the West African tomato-infecting begomoviruses (WATIBs) (Figure 3). Beside the WATIBs, others such as *Tomato yellow leaf curl Mali virus* (TYLCMLV), *Pepper yellow vein Mali virus* (PepYVMLV), and *Tomato leaf curl Mali virus* (ToLCMLV) have been identified infecting tomato and pepper in the region [49-57] (Figure 3 arrows). Apart from the pepper and tomato-infecting begomoviruses, others such as *Cotton leaf curl Gezira virus* (CLCuGeV), *Okra leaf curl Cameroon virus* (OLCuCMV), and *Okra yellow crinkle virus* (OYCrV) have been identified infecting okra in West and Central Africa [49,52,56,58] (Figure 3). Putative recombination events were detected in begomovirus genomes for all three plant species studied, indicating that recombination is an important mechanism for their evolution. The concentration of previous research in the region on ACMD, OLCD, and ToLCD, clearly underscores the importance of these

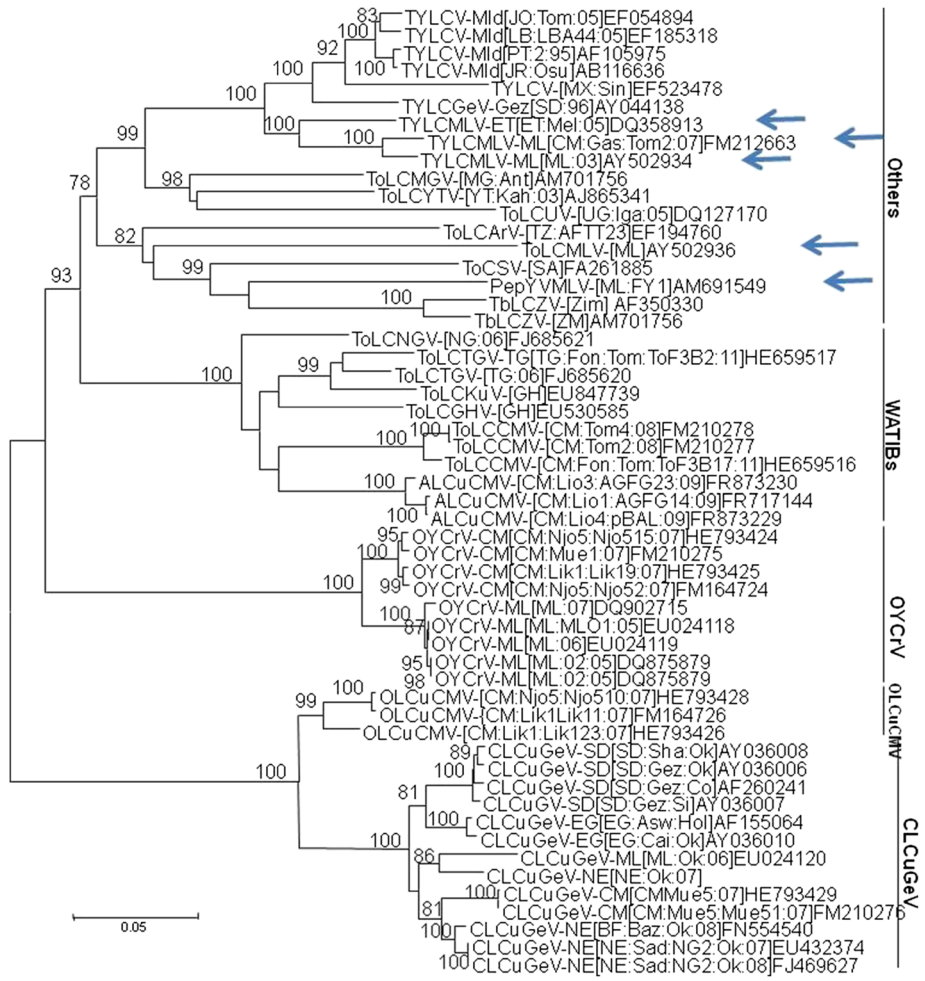

Figure 3 Neighbor-joining phylogenetic analysis. Using MEGA 4.0 of the complete genome of isolates of the West African tomato-infecting begomoviruses (WATIBs) and other begomovirus isolates from the NCBI GenBank. *Arrows* indicate other begomoviruses identified in West and Central Africa other than the WATIBs. *Horizontal lines* are in proportion to the number of nucleotide substitutions per site. *Numbers* represent percent bootstrap values out of 2,000 replicates. The GenBank accession numbers of the nucleotide sequences are indicated. The abbreviations are according to Brown et al. [59].

diseases, which are still being somewhat neglected due to the lack of trained personnel in advanced molecular techniques and adequate funding for fundamental research.

Previously, information on the existence and diversity of DNA satellite molecules associated with monopartite begomoviruses has been mainly available for Asia [60-63]. The presence of DNA satellites in Africa was first shown in a survey for alphasatellites in samples from Asia and Africa, where they were found in different plants from both Egypt and Kenya [29] (Figure 4). Recently, one betasatellite, *Cotton leaf curl Gezira betasatellite* (CLCuGB), initially identified in the Nile basin, has been identified in West and Central Africa, associated with diseased okra and tomato [52,55,56,58,64] (Figure 5). Two new betasatellites, *Ageratum leaf curl Cameroon betasatellite* (ALCCMB) and *Tomato leaf curl Togo betasatellite* (ToLCTGB), have been identified infecting *A. conyzoides* and tomato in Cameroon and Togo, respectively [51,53,65] (Figure 5). Also, new types of alphasatellites, *Ageratum leaf curl Cameroon alphasatellite* (ALCCMA), *Tomato leaf curl Cameroon alphasatellite* (ToLCCMA), *Okra leaf curl Mali alphasatellite* (OLCuMLA), *Okra leaf curl Burkina Faso alphasatellite* (OLCuBFA), and *Okra yellow crinkle alphasatellite* (OYCrA), have been identified infecting *A. conyzoides*, okra, and tomato [50-53,56,58] in West Africa and the lone Central African state, Cameroon.

In West and Central Africa, *Bemisia tabaci* has been associated with suspected begomovirus infections in many crop species, including cassava, bean, cotton, eggplant, pepper, squash, tomato, okra, and watermelon as well as weeds of the genera *Ageratum*, *Asystasia*, *Clerodendrum*, *Emilia*, and *Malvastrum*. This observation was based on the consistent presence of *B. tabaci* on plants exhibiting characteristic symptoms of begomovirus infection (leaf

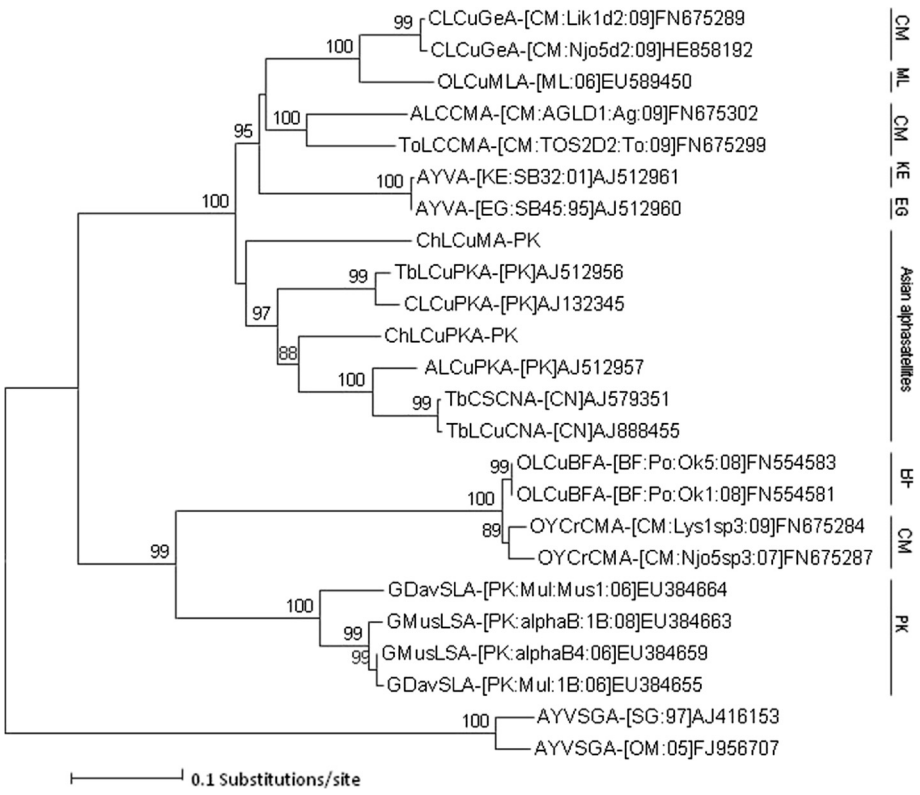

Figure 4 Neighbor-joining analysis. Using MEGA 4.0 of the complete genome of isolates of the West and Central African alphasatellites and other alphasatellite isolates from GenBank. *Horizontal lines* are in proportion to the number of nucleotide substitutions per site. *Numbers* represent percent bootstrap values out of 2,000 replicates. The GenBank accession numbers of the nucleotide sequences of alphasatellites are indicated. The abbreviations are according to Briddon et al. [66].

curling and distortion, green or yellow foliar mosaic, stunting, reduced yields). Thus, there is a pressing need for additional information on the diversity and distribution of begomoviruses and satellites in vegetable crops and/or dicotyledonous weeds, which likely serve as virus reservoirs. This review thus presents a tip of the iceberg on the diversity of begomoviruses and associated satellite DNAs infecting vegetable crops in West and Central Africa.

Economic impact of begomoviruses/ssDNA satellites on vegetable production systems and food security in West and Central Africa

Vegetables have been grown and utilized traditionally in most if not all of the African countries for home consumption and domestic markets. Of late, vegetable production has shifted from subsistence to include export markets representing an important driver for growth due to employment opportunities in production, processing, and trade. Vegetables have proven to be important rain-fed crops [67], and several advantages and potentials of vegetable production are still not being fully exploited.

With the rapid urbanization and population growth, market-oriented vegetable production is increasing in peri-urban areas and has considerable potential for earning foreign exchange, thus generating employment opportunities and income, improving food security, alleviating poverty, and enhancing development in the region. Vegetables account for an estimated 40% of the market sales of products in the region [68]. Vegetables are mainly grown and traded by women in domestic markets with substantial regional market linkages [68]. Increased and sustained growth in this sector will translate into more income for women and associated benefits for household nutrition and food security, health, and educational status of children [68].

Based on the Food and Agriculture Organization (FAO) statistics, annual production of okra, pepper and tomato in WCA fluctuates over the years [68]. The region accounts for more than 75% of okra produced in Africa and West Africa is the largest okra producer [69] (Figure 6). Okra production has increased moderately in Central Africa from 9.4 thousand tons in the year 2000 to 23 thousand tons in 2004. From 2004, okra experienced a decrease of 61% before continuing its slow increase over years. Pepper and tomato production was more important in West Africa than in Central Africa. Pepper and tomato production in WCA has increased moderately over the years from 2000 to 2012. Pepper production in

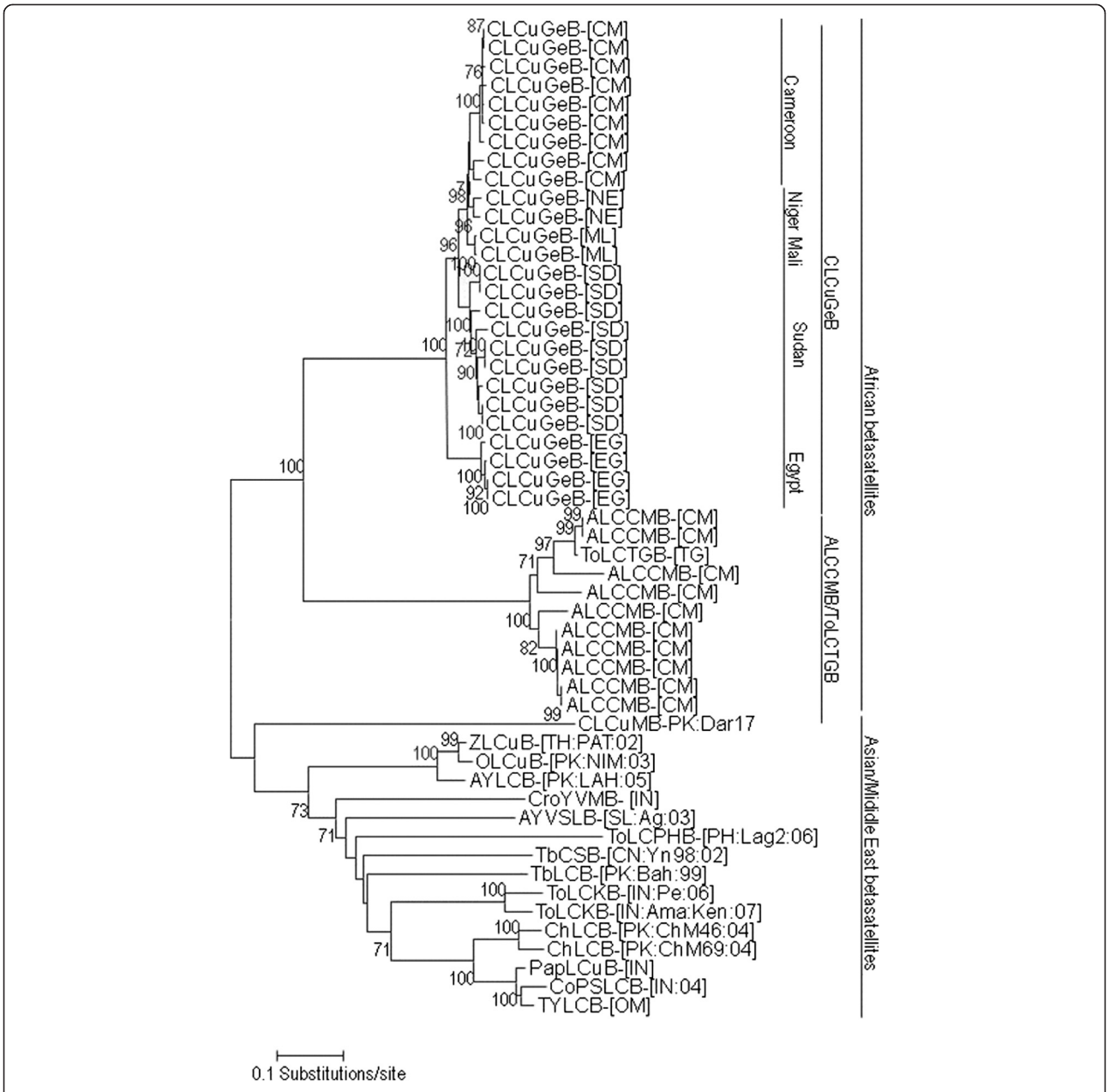

Figure 5 Neighbor-joining phylogenetic analysis. Using MEGA 4.0 of the complete genome of isolates of the West and Central African betasatellites and other betasatellite isolates from the NCBI GenBank. *Horizontal lines* are in proportion to the number of nucleotide substitutions per site. *Numbers* represent at the branch nodes are percent bootstrap values out of 2,000 replicates. The abbreviations are according to Briddon et al. [66].

West Africa increased from 2000 to 2008 before decreasing from 2009 up to 2013 while in Central Africa tomato production growth started in 2009 (Figure 6). Yields of okra, tomato and pepper in WCA as depicted in Figure 6 are lagging far behind those worldwide. In fact, much of the increase in world vegetable production is attributed mainly to yield improvements and their yield is still way below their maximum potential affecting the production which experienced rising infections by begomoviruses and associated ssDNA satellites [70,71]. As early as the 1950s, begomoviruses have been reported to cause important losses in vegetables ranging from 20% up to 100% [70-72].

Potential for control

The sustainable management of begomovirus diseases in West and Central Africa will depend on the use of an integrated pest management (IPM) approach. The use of resistant/tolerant cultivars is an important part of disease

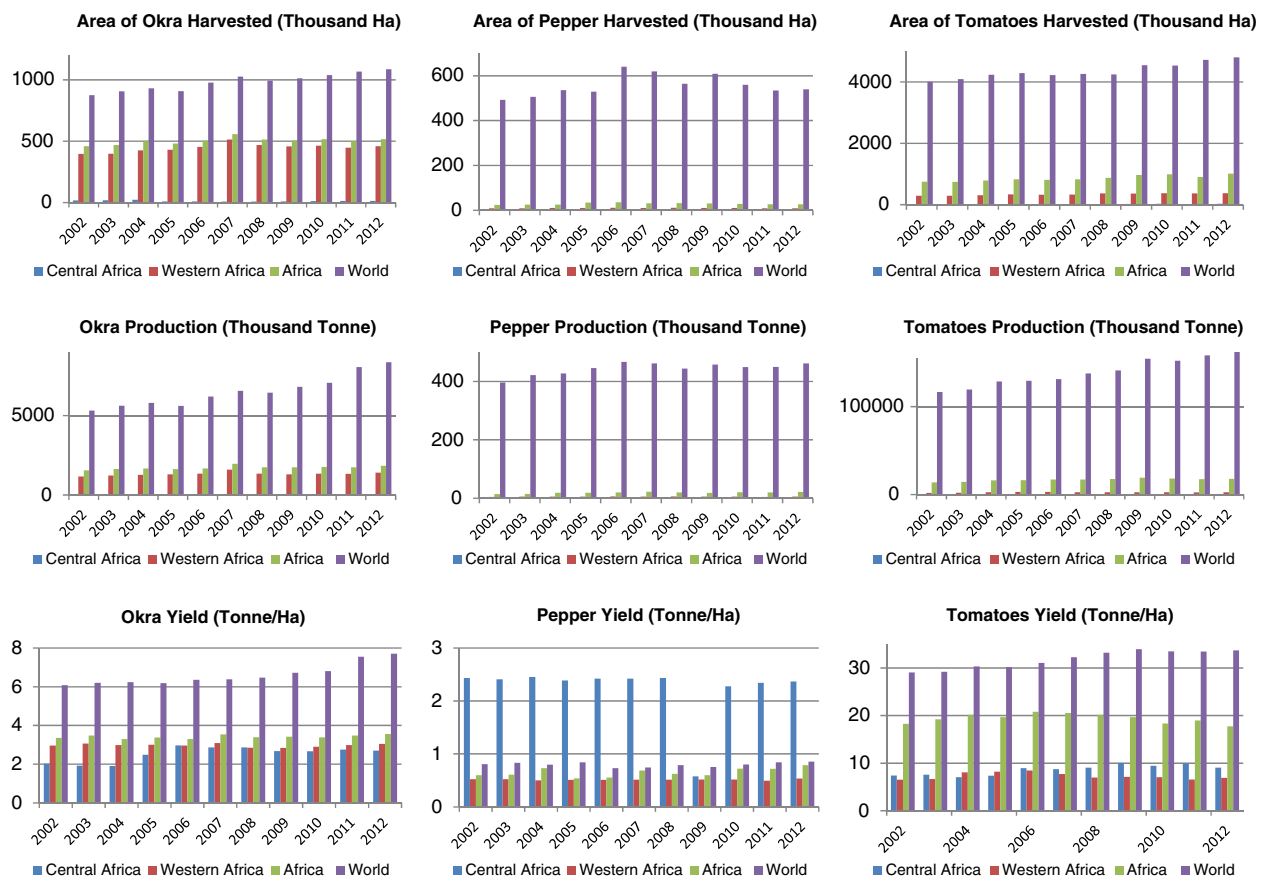

Figure 6 Production and yield trends for okra, pepper, and tomato in West and Central Africa (WCA).

control as well as the use of different methods for limiting begomovirus spread by the whitefly vector. However, the breeding for resistance to begomoviruses is complicated by their high diversity, ability to form new genotypes by recombination, and the occurrence of DNA satellites.

Resistance

The local tomato cultivars in West and Central Africa as well as old cultivars such as Moneymaker and Roma are susceptible to important diseases, including tomato leaf curl/tomato yellow leaf curl (ToLCD/TYLCD) [73,74]. Recently, tomato cultivars with resistance to begomoviruses have been widely adopted throughout the world. They are not immune to infection but may give an acceptable yield even when infected [75,76]. The resistance has been crossed into tomato from wild relatives [77]. Common resistance genes in tomato are the allelic genes *Ty-1* and *Ty-3*, which originate from accessions of *Solanum chilense*. These resistance genes have been cloned and found to encode an RNA-dependent RNA polymerase, and the resistance involves increased cytosine methylation of the viral genome [75]. Field trials carried out in Mali under a high infection pressure of begomoviruses showed that breeding lines of tomato with the resistance genes *Ty-1* and *Ty-3* produced a higher yield than the commercial susceptible cultivars [78]. In a recent survey, 41 tomato varieties from different sources were screened in the field in Senegal for resistance to TYLCD [74]. The trial included also commercial varieties with resistance or tolerance to TYLCD. A substantial variability was observed with a disease incidence from 0% to 100% and a severity of 0% to 89%. Twelve genotypes were identified as having a high level of resistance and being suitable for cultivation under a high disease pressure. Similar studies were carried out simultaneously in Benin, Burkina Faso, Ghana, Mali, and Togo, using the same varieties, but the varieties turned out to perform differently in the different countries [74]. It will be important to identify the factors causing this difference in resistance, and one factor would likely be the viruses. The screens were completely based on symptoms, and there could have been infection with different begomoviruses and DNA satellites as well as with other viruses. The resistance conferred by the gene *Ty-1* has, for example, been compromised when plants become infected with multiple RNA viruses [75]. Also in pepper, there are variations in the susceptibility to begomovirus

infections. In Nigeria, moderate resistance in four pepper cultivars has been identified [79]. In addition to the conventional breeding, different transgenic approaches for achieving resistance or tolerance have been tested against begomoviruses infecting tomato and pepper [80-82] and this may be a way forward for obtaining durable resistance.

In West and Central Africa, leaf curl disease is regarded as the most important biotic stress for okra, and there is a lack of viral resistance/tolerance [58,83]. However, okra has been regarded as a crop of minor importance, and the breeding efforts for okra in the region have been limited.

In India and Bangladesh, screens have revealed resistance and tolerance to be present in some cultivated varieties and wild species of okra against yellow vein mosaic disease (OYVMD), which is also caused by begomoviruses [84]. The genetic material identified in these screens may be of value also for adoption in breeding programs for resistance/tolerance to begomoviruses present in Africa. Screens of genetic material in the region will probably also reveal useful breeding material [58,83]. In a field trial in Burkina Faso with four accessions of a local okra cultivar and four improved commercial okra cultivars, the incidence of OLCD was higher in the local cultivar compared to the commercial cultivars [58]. The difference was suggested to be related to a possible resistance in the commercial cultivars to the whitefly vector.

Whitefly vector management

Insecticides are frequently used to control *B. tabaci* in different crops, and it is often the main way of control. However, besides the detrimental effects on human health and the environment, the repeated use of the same types of insecticides will lead to the selection for resistance in the insect and may also reduce the numbers of beneficial insects which are natural enemies of whiteflies. Cotton is heavily sprayed for management of insects, and this practice also affects the control of whiteflies in vegetable crops. Tests on *B. tabaci* populations from cotton fields in Burkina Faso showed that they were resistant to the recommended doses of several chemicals, suggesting that selection for resistance was likely to occur [85]. The systematic use in cotton of pyrethroids, organophosphates and neonicotinoids has probably lead to the observed reduction in susceptibility or even resistance in populations of *B. tabaci* in Benin, Togo and Burkina Faso [86]. Transgenic cotton varieties with *Bacillus thuringiensis* (Bt) resistance against lepidopteran larvae have been introduced into Burkina Faso. The cultivation of these varieties involves less insecticide with a reduction in the amount of chemicals used. However, neonicotinoids are applied to the Bt cotton for control of whiteflies, and this leads to the selection of the resistant Q-biotype of *B. tabac*i [86,87]. Therefore, it is important that insecticides are managed in a sustainable manner and in combination with other control methods.

Agricultural practices

In West and Central Africa, tomato is produced all around the year, which aggravates the problem with diseases. ToLCD/TYLCD is a large constraint, especially during the dry season, when the whitefly populations are highest [54]. The implementation of host-free periods has been a successful way for control of begomovirus infections of tomato, for example, in Israel and the Dominican Republic [88,89]. A host-free period with no tomato or solanaceous crop is also used in some areas of West Africa with a high incidence of ToLCD/TYLCD [78,90]. The establishment of such a period can be difficult to implement since our knowledge of alternative hosts of begomoviruses is very limited and may lead to an associated reduction in farmers' income because they will be out of production during this period. Still, in Mali, a host-free period of 2 months has been reported to be successful for managing begomovirus infections of tomato [90]. As part of an IPM approach, other agricultural practices for management of ToLCD/TYLCD and other diseases caused by begomoviruses include eradication of source plants, cultivation of bait plants, reflective mulches and inclusion of physical barriers, and use of virus-free transplants [89-92]. In Nigeria, early planting of pepper or tomato has been found to reduce disease incidence [79]. Practices suggested for vegetable production in tropical Africa have also been intercropping to divert whiteflies or changed sowing times to avoid periods of peaks with pest populations [93]. The best-suited practices for control of begomovirus infections will depend on the local conditions, and different types of management methods will have to be tested.

Virus reservoirs

Besides the crop plants, the begomoviruses have weeds and wild plants as hosts. For complete understanding of the epidemiology and for developing appropriate control measures, the identification of alternative hosts is an important aspect of the study which has been somewhat neglected in the past. Presently, the begomovirus reservoirs remain largely unknown. A recent study showed that the common weed *A. conyzoides* in Cameroon is host to a complex consisting of *Ageratum leaf curl Cameroon virus* (ALCCMV), *Ageratum leaf curl betasatellite* (ALCCMB), and *Ageratum leaf curl Cameroon alphasatellite* (ALCCMA) [51]. Sequence analyses revealed that the begomovirus was most closely related to a group of tomato-infecting begomoviruses from West Africa suggesting that these viruses may have common hosts. The betasatellite of *A. conyzoides* has now also been detected in tomato [53]. Of late, a new betasatellite, *Tomato leaf curl Togo betasatellite* (ToLCTGB), has

been identified infecting tomato in Togo and with its closest relative being ALCCMB [65]. That uncultivated (wild) hosts serve as reservoirs for these viruses are further demonstrated by the recent identification of *Lamium amplexicaule* (family Lamiaceae) as a host of *Tomato yellow leaf curl virus* (TYLCV) in Korea [94]. These results further stress the urgent need for the search of alternative hosts of begomoviruses and associated ssDNA satellites infecting vegetables in West and Central Africa.

Whitefly *B. tabaci* vector: biological and genetic variability

The whitefly *B. tabaci* (Genn.) sibling species group [95] is a group of morphologically indistinguishable haplotypes that exhibit different biological characteristics, including host range, fecundity, dispersal behavior, virus transmission efficiency, and insecticide resistance [96]. Regardless of these and other phenotypic differences between certain haplotypes or biotypes (well-defined) [96-98], as a group, they are the only insect vectors of the genus, *Begomovirus*, worldwide. These differences and similarities can have important bearings on the epidemiology of begomovirus-incited diseases of vegetable, fruit, and fiber crops [96,99], and in some instances are known to drive begomoviral diversification [100]. The size of a whitefly vector population is often directly associated with begomovirus disease incidence, including a number of outbreaks [44,47,50,52,54,56,64,101,102] and epidemics in Africa such as the CMD pandemic in East and Central Africa [103]. However, in some instances, extremely high incidence has been observed even when population levels are low.

The overall genetic diversity of the *B. tabaci* sibling species group in western Africa is not well studied. Burban et al. [104] first showed host-associated differences among populations of *B. tabaci* from cassava and okra and other host plants in Côte d'Ivoire, based on isozyme electrophoresis and experimental host range studies. This report was corroborated by the presence of cassava and non-cassava haplotypes based on RAPD-PCR markers and the internal transcribed spacer-1 (ITS-1) [105]. The haplotype colonizing okra and other vegetable plant species appears to be polyphagous, whereas the cassava-associated haplotype was found colonizing only cassava plants. Gueguen et al. [106] reported three haplotypes in Burkina Faso, a sub-Saharan type, a silvering type (B-like clade), and a close relative of the Spanish Q (Q-like clade) [96,98,99]. Recently, Gnankiné et al. [107] sampled *B. tabaci* populations in 20 locations and seven cultivated plant hosts in Burkina Faso, Benin, and Togo and obtained a result similar to the latter study. However, they additionally demonstrated evidence for host and geographical affiliations, in that, in Benin and Togo, the B-like, silvering and sub-Saharan African non-silvering types predominated. In contrast, Q-like haplotypes were most widespread in Burkina Faso. In some instances the B-like silvering and Q-like haplotypes overlapped in host and location. In cassava plantings, an invasive-like non-B/non-Q type that also differs genetically from the sub-Saharan non-silvering type found in the above three countries, has been reported accompanying the rapidly spreading CMD, as it appears to be spreading toward and/or into Cameroon and neighboring countries [108].

Diagnostic approaches for begomoviruses

The majority of approaches currently being used for begomovirus epidemiological and other studies rely nearly entirely on molecular methods. A number of DNA extraction methods have been used to isolate and purify total genomic DNA from plant leaves, stems, and roots/tubers suspected to harbor begomoviruses. Most commonly, the CTAB method of Doyle and Doyle [109] is used along with a variety of its modifications and methods optimized for difficult-to-handle plants [110]. Manufactured kits tailored for isolation of total genomic DNA from plants are also widely implemented. The method of choice is best selected based on the characteristics of the plant host from which total genomic DNA will be isolated. Plant sap from leaves or other parts of begomoviral-infected plants have been successfully applied to and the total genomic DNA eluted from FTA cards. This has been shown to be effective for short-term storage of total genomic DNA that is amenable to PCR amplification of viral DNA fragments [111], or as template for RCA of begomoviral circular DNA genomes [112].

PCR [113] is a widely used method for the initial amplification of begomoviral genomic fragments to demonstrate begomoviral presence. Several broad-spectrum primer pairs have been designed that are useful in many instances, but thus far, no single pair of primers can be guaranteed to amplify all begomoviruses given the extensive genomic variation occurring within the genus worldwide. The most common PCR target is the begomoviral coat protein (CP) gene, or a region of it that is accessible for most isolates using degenerate PCR primers that target the middle, or 'core' region, of the ORF, designated AC1048/AV494 [114,115]. Modified degenerate core CP primers have been designed to have more broad-spectrum capabilities. They are based on the substantially greater number of sequences available in the GenBank database by 2011 and are available upon request (J.K. Brown, personal communication). The CP gene has proven to be an ideal target because it is relatively highly conserved across the genus. Two other sets of primers with broad-spectrum amplification of bipartite begomoviruses that amplify fragments of both the DNA-A and DNA-B components have been

reliable for PCR amplification of primarily the Western Hemisphere begomoviruses [116] and some bipartite viruses from the Eastern Hemisphere. A third set also primarily useful for PCR amplification of bipartite begomoviruses targets conserved regions in the intergenic/common region and the Rep gene [117]. Immuno-capture PCR has also been used successfully when an antibody to the virus or related viruses is available [118]. Universal primers [119,120] have been designed to facilitate PCR amplification, cloning, and sequencing of betasatellite molecules that associate with a number of monopartite begomoviruses. They are particularly prevalent among begomoviruses endemic to Africa, Asia, and Latin America and most commonly are essential for infectivity and/or pathogenicity of the 'helper' begomovirus, encoding a single multifunctional protein that functions as a suppressor of host gene silencing among others.

Recently, the most widely applied method for detecting and cloning begomoviral genomes is the non-sequence-specific approach that uses bacteriophage phi29 DNA polymerase [121,122], now referred to as RCA [123]. In virology research, it was used first for the detection of papillomaviruses [123]. It was first applied to begomoviruses by Inoue-Nagata et al. [124] and since has become routinely used for begomoviral diagnostics and molecular cloning of whole viral genomes to facilitate their characterization and enable the relatively efficient construction of infectious clones [125] and many others. RCA also has been used to enrich for and prepare template for begomoviral deep sequencing employing next-generation approaches such as Illumina [126].

The most important considerations for implementing RCA for begomovirus diagnostics are to avoid repeated freezing and drying of the DNA so that breaks do not occur in the circular template DNA strands [123]. The amount of input DNA required is variable, depending on the source of the template, but in general, only a small amount of circular DNA (~1 ng) needs to be available for RCA. The most common approach for confirming that the amplification step has produced viral fragments or genomic components of interest is to digest the multimeric high molecular weight product with one or more diagnostic restriction enzymes that are known to or might yield a full-length genome of a particular sized diagnostic fragment. The endonuclease digested dsDNA products are readily visualized by gel electrophoresis in 0.7%–1.2% agarose, with the concentration adjusted to accommodate the range of fragment sizes. Further processing of RCA products including cloning, direct sequencing or additional PCR amplification is carried out. Additional PCR amplification using virus or group-specific primers can be carried out using the RCA product as template when the method is used to enrich for or pre-amplify viral circular DNA. A number of protocols have been published, and some of these are summarized in [123].

Conclusions

With this high diversity, recombination potential, limited knowledge of alternative hosts, and transmission by *B. tabaci*, begomoviruses and their associated ssDNA satellites pose a serious threat to crop production and thus food security in West and Central Africa.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

WNL led the review process, did background literature studies, structured the concepts, developed the arguments and wrote most of the manuscript. DBM wrote the section on economic impact; JKB reviewed the manuscript, helped design the arguments and wrote parts of the manuscript, AK developed the section on potential for control. All authors read and approved the final manuscript.

Authors' information

WNL is the formal National Scientific Coordinator of annual crops research at IRAD, was a visiting scientist at IITA, and presently a Fulbright scholar at Delaware State University, USA, with expertise in molecular plant DNA viruses. DBM is a regional economist at IITA. JKB is Professor, School of Plant Sciences, University of Arizona, Tucson, Arizona, USA, with expertise in plant-infecting DNA viruses, vector biology, and whitefly molecular systematics. AK is a Professor of plant virology at the Department of Plant Biology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.

Acknowledgements

We sincerely thank the anonymous referees for useful comments that strengthened this review.

Author details

[1]International Institute of Tropical Agriculture (IITA), PMB 5320, Oyo road, Ibadan, Nigeria. [2]Institute of Agricultural Research for Development (IRAD), P.O. Box 2123, Messa, Yaoundé, Cameroon. [3]School of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA. [4]Department of Plant Biology, Swedish University of Agricultural Sciences, Linnean Center for Plant Biology, Uppsala BioCenter, P.O. Box 7080, 75007 Uppsala, Sweden.

Received: 5 August 2014 Accepted: 16 December 2014
Published online: 21 January 2015

References

1. Schippers RR. African Indigenous Vegetables. An overview of the Cultural Species. Chatham, UK: Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation; 2000. p. 214.
2. Saunders K, Bedford ID, Yahara T, Stanley J. Aetiology: the earliest recorded plant virus disease. Nature. 2003;422:831.
3. Storey HH, Nichols RW. Studies of the mosaic diseases of cassava. Ann Appl Biol. 1938;25:790–806.
4. Legg JP, Fauquet CM. Cassava mosaic geminiviruses in Africa. Plant Mol Biol. 2004;56:585–99.
5. Salati R, Nahkla MK, Rojas MR, Guzman P, Jaquez J, Maxwell DP, et al. Tomato yellow leaf curl virus in the Dominican Republic: characterization of an infectious clone, virus monitoring in whiteflies, and identification of reservoir hosts. Phytopathology. 2002;92:487–96.
6. Varsani A, Navas-Castillo J, Moriones E, Hernández-Zepeda C, Idris A, Brown JK, et al. Establishment of three new genera in the family *Geminiviridae*: *Becurtovirus*. Eragrovirus and Turncurtovirus Arch Virol. 2014;159:2193–203.
7. Rojas MR, Hagen C, Lucas WJ, Gilbertson RL. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. Annu Rev Plant Physiol Plant Mol Biol. 2005;43:361–94.
8. Stanley J, Bisaro DM, Briddon RW, Brown JK, Fauquet CM, Harrison BD, et al. Geminiviriae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA,

editors. VIIIth Report of the International Committee on Taxonomy of Viruses. Virus Taxonomy. London: Elsevier/Academic Press; 2005. p. 1163–9.
9. Thresh JM, Otim-Nape GW, Legg JP, Fargette D. African cassava mosaic virus disease: the magnitude of the problem. Afri J Root Tub Crops. 1997;2:13–9.
10. Eagle PA, Orozco BM, Hanley-Bowdoin L. A DNA-sequence required for geminivirus replication also mediates transcriptional regulation. Plant Cell. 1994;6:1157–70.
11. Padidam M, Beachy RN, Fauquet CM. The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. Virology. 1996;224:390–404.
12. Harrison BD, Robinson DJ. Green shoots of geminivirology. Physiol Mol Plant Pathol. 2002;60:215–8.
13. Harrison BD, Robinson DJ. Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). Annu Rev Phytopathol. 1999;37:369–98.
14. Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, Robertson D. Geminiviruses: models of plant DNA replication, transcription, and cell cycle regulation. Crit Rev Plant Sci. 1999;18:71–106.
15. Sanderfoot AA, Ingham DJ, Lazarowitz SG. A viral movement protein as a nuclear shuttle. Plant Physiol. 1996;110:23–3.
16. Navot N, Pichersky E, Zeidian M, Zamir D, Czosnek H. *Tomato yellow leaf curl virus*: a whitefly-transmitted geminivirus with a single genomic component. Virology. 1991;185:151–61.
17. Mayo MA, Leibowitz MJ, Palukaitis P, Scholthof KBG, Simon AE, Stanley J, et al. Satellites. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. VIIIth Report of the International Committee on Taxonomy of Viruses. Virus Taxonomy. London: Elsevier/Academic Press; 2005. p. 1163–9.
18. Schneider LR. Satellite-like particle of tobacco ringspot virus that resembles tobacco ringspot virus. Science. 1969;166:1627–9.
19. Roossinck MJ, Sleat D, Palukaitis P. Satellite RNAs of plant viruses: structures and biological effects. Microbiol Rev. 1992;56:265–79.
20. Dry I, Krake LR, Rigden JE, Rezaian MA. A novel subviral agent associated with a geminivirus: the first report of a DNA satellite. Proc Natl Acad Sci U S A. 1997;94:7088–93.
21. Behjatnia SAA, Dry IB, Rezaian MA. Identification of the replication-associated protein binding domain within the intergenic region of tomato leaf curl geminivirus. Nucleic Acids Res. 1998;26:925–31.
22. Saunders K, Bedford ID, Briddon RW, Markham PG, Wong SM, Stanley J. A unique virus complex causes Ageratum yellow vein disease. Proc Natl Acad Sci U S A. 2000;97:6890–5.
23. Tan HNP, Wong SM, Wu M, Bedford ID, Saunders K, Stanley J. Genome organization of *Ageratum yellow vein virus*, a monopartite whitefly-transmitted geminivirus isolated from a common weed. J Gen Virol. 1995;76:2915–22.
24. Stanley J, Saunders K, Pinner MS, Wong SM. Novel defective interfering DNAs associated with ageratum yellow vein geminivirus infection of *Ageratum conyzoides*. Virology. 1997;239:87–96.
25. Liu Y, Robinson DJ, Harrison BD. Defective forms of cotton leaf curl virus DNA-A that have different combinations of sequence deletion, duplication, inversion and rearrangement. J Gen Virol. 1998;79:1501–8.
26. Briddon RW, Mansoor S, Bedford ID, Pinner MS, Markham PG. Clones of cotton leaf curl geminivirus induced symptoms atypical of cotton leaf curl disease. Virus Genes. 2000;20:17–24.
27. Briddon RW, Mansoor S, Bedford ID, Pinner MS, Saunders K, Stanley J, et al. Identification of DNA components required for induction of cotton leaf curl disease. Virology. 2001;285:234–43.
28. Mansoor S, Khan SH, Bashir A, Saeed M, Zafar Y, Malik KA, et al. Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. Virology. 1999;259:190–9.
29. Briddon RW, Bull SE, Amin I, Mansoor S, Bedford ID, Rishi N, et al. Diversity of DNA 1: a satellite-like molecule associated with monopartite begomovirus-DNA β complexes. Virology. 2004;324:462–74.
30. Jose J, Usha R. Bhendi yellow vein mosaic disease in India is caused by association of a satellite with a begomovirus. Virology. 2003;305:310–7.
31. Stenger DC, Revington GN, Stevenson MC, Bisaro DM. Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. Proc Natl Acad Sci U S A. 1991;88:8029–33.
32. Zhou X, Xie Y, Tao X, Zhang Z, Li Z, Fauquet CM. Characterization of DNAβ associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. J Gen Virol. 2003;84:237–47.
33. Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, et al. Diversity of DNA-β, a satellite molecule associated with some monopartite begomoviruses. Virology. 2003;312:106–21.
34. Yang J-Y, Iwasaki M, Machinda C, Machinda Y, Zhou X, Chua N-H. βC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. Genes Dev. 2008;22:2564–77.
35. Tao X, Zhou X. A modified viral satellite DNA that suppresses gene expression in plants. Plant J. 2004;38:850–60.
36. Saunders K, Stanley J. A nanovirus-like component associated with yellow vein disease of *Ageratum conyzoides*: evidence for inter-family recombination between plant DNA viruses. Virology. 1999;264:142–52.
37. Mansoor S, Amin I, Hussain M, Zafar Y, Bull S, Briddon RW, et al. Association of a disease complex involving a begomovirus, DNA1 and a distinct DNA beta with leaf curl disease of okra in Pakistan. Plant Dis. 2001;85:922.
38. Mubin M, Briddon RW, Mansoor S. Complete nucleotide sequence of chilli leaf curl virus and its associated satellites naturally infecting potato in Pakistan. Arch Virol. 2009;154:365–8.
39. Gronenborn G. Nanoviruses: genome organization and protein function. Vet Microbiol. 2004;98:103–10.
40. Huang C, Xie Y, Zhou X. Efficient virus-induced gene silencing in plants using a modified geminivirus DNA1 component. Plant Biotechnol J. 2009;7:254–65.
41. Patil BL, Fauquet CM. Differential interaction between cassava mosaic geminiviruses and geminivirus satellites. J Gen Virol. 2010;91:1871–82.
42. Paprotka T, Metzler V, Jeske H. The first DNA 1-like α satellites in association with New World begomoviruses in natural infections. Virology. 2010;404:148–57.
43. Romay G, Chirinos D, Geraud-Pouey F, Desvies C. Association of an atypical alphasatellite with a bipartite New World begomovirus. Arch Virol. 2010;155:1843–7.
44. Nguessan KP, Fargette D, Fauquet C, Thouvenel JC. Aspects of the epidemiology of okra leaf curl virus in Cote d'Ivoire. Trop Pest Man. 1992;38:122–6.
45. Fargette D, Jeger M, Fauquet CM, Fishpool LDC. Analysis of temporal disease progress of African cassava mosaic virus. Phytopathology. 1993;84:91–8.
46. Swanson MM, Harrison BD. Properties, relationships and distribution of cassava mosaic geminiviruses. Trop Sci. 1994;34:15–25.
47. Konate G, Barro N, Fargette D, Swanson MM, Harrison BD. Occurrence of whitefly-transmitted geminiviruses in crops in Burkina-Faso, and their serological detection and differentiation. Ann Appl Biol. 1995;126:121–9.
48. Czosnek H, Laterrot H. A worldwide survey of tomato yellow leaf curl viruses. Arch Virol. 1997;142:1391–406.
49. Leke WN, Ramsell JNE, Njualem DK, Titanji VPK, Legg JP, Fondong VN, et al. FTA technology facilitates detection and identification of begomoviruses from okra plants in Cameroon. Afri Crop Sci Soc Conf Proc. 2007;8:655–60.
50. Leke WN, Kvarnheden A, Ngane EB, Titanji VPK, Brown JK. Molecular characterization of a new begomovirus and divergent alphasatellite from tomato in Cameroon. Arch Virol. 2011;156:925–8.
51. Leke WN, Brown JK, Ligthart ME, Sattar MN, Njualem DK, Kvarnheden A. *Ageratum conyzoides*: A host to a unique begomovirus disease complex in Cameroon. Virus Res. 2012;163:229–37.
52. Leke WN, Sattar MN, Ngane EB, Ngeve JM, Kvarnheden A, Brown JK. Molecular characterization of begomoviruses and DNA satellites associated with okra leaf curl disease in Cameroon. Virus Res. 2013;174:116–25.
53. Leke WN, Kvarnheden A. Mixed infection of two West African tomato-infecting begomoviruses and Ageratum leaf curl Cameroon betasatellite infecting tomato in Cameroon. Arch Virol. 2014;159:3145–8.
54. Zhou Y-C, Noussourou M, Kon T, Rojas MR, Jiang H, Chen L-F, et al. Evidence of local evolution of tomato-infecting begomovirus species in West Africa: characterization of *Tomato leaf curl Mali virus* and *Tomato yellow crumple virus* from Mali. Arch Virol. 2008;153:693–706.
55. Chen L-F, Rojas M, Kon T, Gamby K, Xoconostle-Cazares B, Gilbertson RL. A severe symptom phenotype in tomato in Mali is caused by a reassortant between a novel recombinant begomovirus (*Tomato yellow leaf curl Mali virus*) and a betasatellite. Mol Plant Pathol. 2009;10:415–30.
56. Kon T, Rojas MR, Abdourhamane IK, Gilbertson RL. Roles and interactions of begomoviruses and satellite DNAs associated with okra leaf curl disease in Mali. West Africa J Gen Virol. 2009;90:1001–13.

57. Lett JM, Lefeuvre P, Couston L, Hoareau M, Thierry M, Reynaud B, et al. Complete genomic sequences of *Tomato yellow leaf curl Mali virus* isolates infecting tomato and pepper from the North Province of Cameroon. Arch Virol. 2009;154:535–40.
58. Tiendrébéogo F, Lefeuvre P, Hoareau M, Villemot J, Konate G, Traoré AS, et al. Molecular diversity of *Cotton leaf curl Gezira virus* isolates and their satellite DNAs associated with okra leaf curl disease in Burkina Faso. Virol J. 2010;7:48.
59. Brown JK, Fauquet CM, Briddon RW, Zerbini M, Moriones E, Navas-Castillo J. Family *Geminiviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus Taxonomy: Classification and Nomenclature of Viruses—Ninth Report of the International Committee on Taxonomy of Viruses. USA: Elsevier Academic Press; 2012. p. 351–73.
60. Bull SE, Tsai W-S, Briddon RW, Markham PG, Stanley J, Green SK. Diversity of begomovirus DNA β satellites of non-malvaceous plants in east and south east Asia. Arch Virol. 2004;149:1193–200.
61. Nawaz-ul-Rehman MS, Fauquet CM. Evolution of geminiviruses and their satellites. FEBS Lett. 2009;583:1825–32.
62. Sivalingam PN, Malathi VG, Varma A. Molecular diversity of the DNA-β satellites associated with tomato leaf curl disease in India. Arch Virol. 2010;155:757–64.
63. Briddon RW, Stanley J. Subviral agents associated with plant single-stranded DNA viruses. Virology. 2006;344:198–210.
64. Shih SL, Kumar S, Tsai WS, Lee LM, Green SK. Complete nucleotide sequences of okra isolates of *Cotton leaf curl Gezira virus* and their associated DNA-β from Niger. Arch Virol. 2009;154:369–72.
65. Kon T, Gilbertson RL. Two genetically related begomoviruses causing tomato leaf curl disease in Togo and Nigeria differ in virulence and host range but do not require a betasatellite for induction of disease symptoms. Arch Virol. 2012;157:107–20.
66. Briddon RW, Ghabrial S, Lin N-S, Palukaitis P, Scholthof K-BG, Vetten H- J. Satellites and other virus-dependent nucleic acids. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus Taxonomy: Classification and Nomenclature of Viruses—Ninth Report of the International Committee on Taxonomy of Viruses. USA: Elsevier Acadmic Press; 2012. p. 1211–9.
67. Pasternak D, Senbeto D, Nikiema A, Kumar S, Fatondji D, Woltering L, et al. Bioreclamation of degraded African lands with women empowerment. Chronica Hortic. 2009;49:24–7.
68. AVRDC. 2014–2016 Medium-term Plan. Shanhua, Taiwan: AVRDC – The World Vegetable Center; 2014. p. 14–776. 123 pp.
69. FAOSTAT. Food and Agricultural Organization of the United Nations. On-line and Multilingual Database, 2014 http://faostat.fao.org.
70. Alvarez PA, Abud-Antún AJ. Reporte de Republica Dominicana. CEIBA (Honduras). 1995;36:39–47.
71. Polston JE, Anderson PK. The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. Plant Dis. 1997;81:1358–69.
72. Caballero R, Rueda A. Las moscas blancas en Honduras. In: Hilje L, Arboleda O, editors. Las moscas blancas (Homoptera: Aleyrodidae) en America Central y El Caribe. Turrialba, Costa Rica: CATIE; 1993. p. 50–3.
73. Fufa F, Hanson P, Dagnoko S, Dhaliwal M. AVRDC – The World Vegetable Center tomato breeding in sub-Saharan Africa: Lessons from the past, present work, and future prospects. Acta Hortic. 2011;911:87–98.
74. Camara M, Mbaye AA, Noba K, Samb PI, Diao S, Cilas C. Field screening of tomato genotypes for resistance to Tomato yellow leaf curl virus (TYLCV) disease in Senegal. Crop Protect. 2013;44:59–65.
75. Butterbach P, Verlaan MG, Dullemans A, Lohuis D, Visser RGF, Bai Y, et al. Tomato yellow leaf curl virus resistance by *Ty-1* involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. Proc Natl Acad Sci U S A. 2014;111:12942–7.
76. Ozores-Hampton M, Stansly PA, McAvoy. Evaluation of round a Roma-type tomato varieties and advanced breeding lines resistant to *Tomato yellow leaf curl virus* in Florida. HortTechnology. 2013;23:689–98.
77. Ji Y, Scott JW, Hanson P, Graham E, Maxwell DP. Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting begomoviruses. In: Czosnek H, editor. Tomato Yellow Leaf Curl Virus Disease. Dordrecht: Springer; 2007. p. 343–62.
78. Dagnoko S, Hanson P, Fufa F, Kollo IA. Preliminary performance of tomato breeding lines for yield, fruit quality and resistance to Tomato yellow leaf curl disease. Acta Hortic. 2011;911:455–68.
79. Alegbejo MD. Whitefly transmitted plant viruses in Nigeria. J Sustain Agr. 2000;17:99–109.
80. Vu TV, Choudhury NR, Mukherjee SK. Transgenic tomato plants expressing artificial microRNAs for silencing the pre-coat and coat proteins of a begomovirus, *Tomato leaf curl New Delhi virus*, show tolerance to infection. Virus Res. 2013;172:35–45.
81. Medina-Hernández D, Rivera-Bustamante R, Tenllado F, Holguín-Penã RJ. Effects and effectiveness of two RNAi constructs for resistance to *Pepper golden mosaic virus* in *Nicotiana benthamiana* plants. Viruses. 2013;5:2931–45.
82. Reyes MI, Nash TE, Dallas MM, Ascencio-Ibáñez JT, Hanley-Bowdoin L. Peptide aptamers that bind to geminivirus replication proteins confer a resistance phenotype to tomato yellow leaf curl virus and tomato mottle virus infection in tomato. J Virol. 2013;87:9691–706.
83. Kumar S, Dagnoko S, Haougui A, Ratnadass A, Pasternak D, Kouame C. Okra (*Abelmoschus* spp.) in West and Central Africa: potential and progress on its improvement. Afr. J Agric Res. 2010;5:3590–8.
84. Shetty AA, Singh JP, Singh D. Resistance to yellow vein mosaic virus in okra: a review. Biol Agric Hortic. 2013;29:159–64.
85. Otoidobiga LC, Vincent C, Stewart KR. Susceptibility of field populations of adult *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and *Eretmocerus* sp (Hymeoptera: Aphelinidae) to cotton insecticides in Burkina Faso (West Africa). Pest Manag Sci. 2002;59:97–106.
86. Houndété TA, Kétoh GK, Hema OSA, Brévault T, Glitho IA, Martin T. Insecticide resistance in field populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in West Africa. Pest Manag Sci. 2010;66:1181–5.
87. Gnankiné O, Bassolé IHN, Chandre F, Glitho I, Akogbeto M, Dabiré RK, et al. Insecticide resistance in *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and *Anopheles gambiae* Giles (Diptera: Culicidae) could compromise the sustainability of malaria vector control strategies in West Africa. Acta Trop. 2013;128:7–17.
88. Ucko O, Cohen S, Ben-Joseph R. Prevention of virus epidemics by a crop-free period in the Arava region of Israel. Phytoparasitica. 1998;26:313–21.
89. Gilbertson RL, Rojas MR, Kon T, Jaquez J. Introduction of Tomato yellow leaf curl virus into the Dominican Republic: the development of a successful integrated pest management system. In: Czosnek H, editor. Tomato Yellow Leaf Curl Virus Disease. Dordrecht: Springer; 2007. p. 279–303.
90. Pfeiffer DG, Mullins DE, Gilbertson RL, Brewster CC, Westwood J, Miller SA, et al. IPM packages developed for vegetable crops in West Africa. Phytopathology. 2011;101:S228.
91. Antignus Y. The management of Tomato yellow leaf curl virus in greenhouses and the open field, a strategy of manipulation. In: Czosnek H, editor. Tomato Yellow Leaf Curl Virus Disease. Dordrecht: Springer; 2007. p. 263–78.
92. Polston JE, Lapidot M. Management of Tomato yellow leaf curl virus: US and Israel perspectives. In: Czosnek H, editor. Tomato Yellow Leaf Curl Virus Disease. Dordrecht: Springer; 2007. p. 251–62.
93. Olasantan FO. Vegetable production in tropical Africa: status and strategies for sustainable management. J Sustain Agr. 2007;30:41–70.
94. Kil E-J, Park J, Lee H, Kim J, Choi H-S, Lee K-Y, et al. *Lamium amplexicaule* (*Lamiaceae*): a weed reservoir for tomato yellow leaf curl virus (TYLCV) in Korea. Arch Virol. 2014;159:1305–11.
95. Brown JK, Bird J, Frohlich DR, Rosell RC, Bedford ID, Markham PG. The relevance of variability within the *Bemisia tabaci* species complex to epidemics caused by Subgroup III geminiviruses. In: Gerling D, Mayer RT, editors. Bemisia '95: Taxonomy, Biology, Damage, Control and Management, Brown JK, Bird J, Frohlich DR, Rosell RC, Bedford ID, Markham PG. Wimborne, UK: Intercept Publications; 2005. p. 77–92.
96. Brown JK. *Bemisia:* phylogenetic biology of the *Bemisia tabaci* sibling species group. In: Stansly PA, Naranjo SE, editors. *Bemisia*: Bionomics and Management of a Global Pest. Dordrecht-Heidelberg-London-New York: Springer; 2010. p. 31–67. 350pp.
97. Frohlich DI, Torres-Jerez ID, Bedford PG, Markham PG, Brown JK. A phylogeographic analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol Ecol. 1999;8:1593–602.
98. Gill R, Brown JK. Systematics of *Bemisia* and *Bemisia* relatives: can molecular techniques solve the *Bemisia tabaci* complex conundrum—a taxonomist's viewpoint. In: Stansly PA, Naranjo SE, editors. *Bemisia*: Bionomics and Management of a Global Pest. Dordrecht-Heidelberg-London-New York: Springer; 2010. p. 5–29. 350pp.
99. Brown JK, Coats S, Bedford ID, Markham PG, Bird J, Frohlich DR. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). Biochem Genet. 1995;33:205–14.

100. Brown JK. The *Bemisia tabaci* complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. APSnet Features. 2007. 10.1094/APSnetFeature-2007-0107.
101. D'Hondt MD, Russo M. Tomato yellow leaf curl in Senegal. Phytopathology. 1985;112:153–60.
102. Fauquet C, Thouvenel J-C. Plant viral diseases in the Ivory Coast. Paris: Editions de 1'ORSTOM; 1987. p. 243.
103. Legg JP, Sseruwagi P, Boniface S, Okao-Okuja G, Shirima R, Bigirimana S, et al. Spatio-temporal patterns of genetic change amongst cassava *Bemisia tabaci* whiteflies driving virus pandemics in East and Central Africa. Virus Res. 2014;186:61–75.
104. Burban C, Fishpool LDC, Fauquet C, Fargette D, Thouvenel J-C. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.), (Hom., Aleyrodidae). J Appl Entomol. 1992;113:416–23.
105. Abdullahi I, Winter S, Atiri GI, Thottappilly G. Molecular characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava. Bull Entomol Res. 2003;93:97–106.
106. Gueguen G, Vavre F, Gnankine O, Peterschmitt M, Charif D, Chiel E, et al. Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. Mol Ecol. 2010;19:4365–78.
107. Gnankiné O, Mouton L, Henri H, Terraz G, Houndeté T, Martin T, et al. Distribution of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotypes and their associated symbiotic bacteria on host plants in West Africa. Insect Conserv Divers. 2013;6:411–21.
108. Legg JP, Sseruwagi P, Boniface S, Okao-Okuja G, Shirima R, Bigirimana S, et al. Spatio-temporal patterns of genetic change amongst cassava *Bemisia tabaci* whiteflies driving virus pandemics in East and Central Africa. Virus Res. 2013;186:61–75.
109. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990;12:13–5.
110. Doyle J. DNA protocols for plants. In: Hewitt GM et al., editors. Molecular Techniques in Taxonomy, Vol 57 NATO ASI Series. Berlin Heidelberg: Springer; 1991.
111. Ndunguru J, Taylor NJ, Yadav J, Aly H, Legg JP, Aveling T, et al. Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. Virol J. 2005;2:45.
112. Haible D, Kober S, Jeske H. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. J Virol Methods. 2006;135:9–16.
113. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988;239:487–91.
114. Brown JK, Idris AM, Torres-Jerez I, Banks GK, Wyatt SD. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. Arch Virol. 2001;146:1–18.
115. Wyatt SD, Brown JK. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology. 1996;86:1288–93.
116. Idris AM, Brown JK. Sinaloa tomato leaf curl geminivirus: biological and molecular evidence for a new subgroup III virus. Phytopathology. 1998;88:648–57.
117. Rojas MR, Gilbertson RL, Russell DR, Maxwell DP. Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted geminiviruses. Plant Dis. 1993;77:340–7.
118. Rampersad SN, Umaharan P. Detection of begomoviruses in clarified plant extracts: a comparison of standard, direct-binding, and immunocapture PCR techniques. Phytopathology. 2003;93:1153–7.
119. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG. Universal primers for the PCR-mediated amplification of DNA β: a molecule associated with some monopartite begomoviruses. Mol Biotechnol. 2002;20:315–8.
120. Bull SE, Briddon RW, Markham PG. Universal primers for the PCR-mediated amplification of DNA1: a satellite-like molecule associated with begomovirus-DNA β complexes. Mol Biotechnol. 2003;23:83–6.
121. Blanco L, Bernad A, Lázaro JM, Martín G, Garmendia C, Salas M. Highly efficient DNA synthesis by the phage phi 29 DNA polymerase. Symmetrical mode of DNA replication. J Biol Chem. 1989;264:8935–40.
122. Dean FB, Nelson JR, Giesler TL, Lasken RS. Rapid amplification of plasmid and phage DNA using Phi 29 DNA polymerase and multiply-primed rolling circle amplification. Genome Res. 2001;11:1095–9.
123. Johne R, Muller H, Rector A, van Ranst M, Stevens H. Rolling-circle amplification of viral DNA genomes using phi29 polymerase. Trends Microbiol. 2009;17:205–11.
124. Inoue-Nagata AK, Albuquerque LC, Rocha WB, Nagata T. A simple method for cloning the complete begomovirus genome using the bacteriophage φ29 DNA polymerase. J Virol Methods. 2004;116:209–11.
125. Wyant PS, Strohmeier S, Schafer B, Krenz B, Assuncao IP, Lima GSD, et al. Circular DNA genomics (circomics) exemplified for geminiviruses in bean crops and weeds of northeastern Brazil. Virology. 2012;427:151–7.
126. Idris A, Al-Saleh M, Piatek MJ, Al-Shahwan I, Ali S, Brown JK. Viral metagenomics: validation of genome enrichment coupled with next generation sequencing reveals reproducibility between laboratory and field samples, and reveals polymorphisms in begomovirus populations from natural plant infections. Viruses. 2014;6:1219.