CHAPTER 2

LITERATURE REVIEW

2.1 CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

2.1.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a basic staple food vital for the livelihood of up to 500 million farmers and countless processors and traders around the world (Plucknett *et al*., 2000). The plant is very robust, resistant to drought and cassava production does not require high inputs. Cassava originated in South America where it was domesticated 2,000-4,000 years B.C. and was introduced into Africa in the 16th century by the Portuguese (Jones, 1959; Fauquet and Fargette, 1990). In the 18th century it was introduced to the east coast of Africa and the Indian Ocean Islands of Zanzibar, Madagascar and R′eunion. The total world cassava production in the year 2002 was 184,852,540 metric tonnes (t), with 100,689,149 t being produced in Africa alone (FAO, 2003). FAO projections are that the global area devoted to cassava by 2005 will be 18.6 million ha, with Africa accounting for 11.9 million ha (FAO, 1997a). Furthermore, it is estimated that the introduction of high-yielding varieties, improved pest and disease control and better processing methods could increase cassava production in Africa by 150% by the year 2015 (FAO, 2000).

2.1.2 Economic importance of cassava

On an energy production basis, cassava is a vital food security crop because it is reliable, producing life sustaining yields when unfavourable climatic conditions cause cereal and pulse crop failures. Cassava produces more food energy per unit of cultivated land than any other staple crop in sub-Saharan Africa (De Bruijn and Fresco, 1989; Plucknett *et al*.,

2000). Cassava is an important staple in the tropics, where it provides a cheap source of dietary carbohydrate energy (720.1 x 10^{12}kJ per day) to over 500 million people (FAO, 2003).

In Africa, the majority of cassava produced is for human consumption (88%) and the remainder is for animal feed (on farm and off-farm) and starch based products (starches and alcohol)(Henry *et al*., 1998). Only a small proportion of African cassava is fed to farm animals, traditionally, mostly in the form of peels. However, a growing interest exists regarding a more commercial approach to cassava incorporation into animal feed mixtures (Plucknett *et al*., 2000).

2.1.3 Cassava production in Tanzania

In Tanzania, cassava is produced mostly by smallholders on marginal or sub-marginal lands. Major producing areas include: the coastal strip along the Indian Ocean (Tanga, Dar es Salaam, Lindi and Mtwara) producing 48.8% of the total cassava crop, around Lake Victoria (Kagera, Mara and Mwanza) (23.7%), Lake Nyasa areas (13.7%), Western zone (7.9%), and Central Zone (5.0%) (Ministry of Agriculture and Cooperative, 2000, unpublished data). Production increased from 4,250,000 MT in 1977 to 6,444,000 metric tonnes in 1997 (FAO, 1997b) and by the year 2002/2003, the cultivated area for cassava was estimated at 664,467,000 ha (MAFS, 2003, unpublished data). Cassava has remained a vital crop for food security and income generation in the country contributing about 25% of cash income in many households (COSCA Tanzania, 1996). Although cassava yields can be quite high, as high as 25 to 40 metric tonnes/ha (Plucknett *et al*., 2000) in Tanzania, yields are generally low (less than five tonnes per hectare) (MDB, 1985), which is far below the world average of 10.5 tonnes /ha and 8.4 metric tonnes /ha for Africa (FAO, 2001).

2.1.4 Uses of cassava in Tanzania

Cassava is important, not only as a food crop, but even more as a major source of income for rural households. As a food crop, cassava has some inherent characteristics, which make it attractive, especially to the smaller farmers. It is rich in carbohydrates that give it many end uses. Cassava provides a basic daily source of dietary energy. Roots are processed into a wide variety of granules, pastes, flours, or consumed freshly boiled or raw (COSCA Tanzania, 1996). The leaves are also consumed as a green vegetable, which provides protein and vitamins A and B. Fresh roots and leaves, because of their perishability, are usually consumed or marketed close to their centres of production. Alternatively it is sold as dried products.

2.1.5 Production constraints

Numerous constraints affect productivity of cassava in Africa. Pests and diseases are widely considered to be the most important constraints to cassava production in Africa. The most significant pests are mealy bug (*Phenacoccus manihoti* Mat. Ferr.) and green spider mite (*Mononychellus tanajoa* (Bondar) (COSCA Tanzania, 1996) both of which were introduced inadvertently to Africa from South America in the early 1970s. The common cassava diseases include cassava bacterial blight [(*Xanthomonas campestris* f.sp. *manihotis* (Berthet and Bondar) Dye], cassava brown leaf spot [(*Cercosporidium henningsii* (Allesch) Deighton] (reviewed by Fauquet and Fargette, 1990) and cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs). After CMD, the next most economically important viral disease of cassava in Tanzania is cassava brown streak disease (CBSD) (Legg and Raya, 1998). CBSD was first reported in Tanzania in 1936 (Storey, 1936) and shown to be endemic in all coastal cassava growing areas up to 1000 meters above sea level (Nichols, 1950; Hillocks, 2003). A virus belonging to the family *Potyviridae*, genus *Ipomovirus* has been identified as the causal agent of CBSD (Monger *et al*., 2001). Cassava brown streak virus (CBSV) induces root symptoms comprising of dry necrotic rotten patches, as well as brown lesions on the stems and

yellowing of leaves (Hillocks *et al*., 1996). Field experiments to determine the effect of the disease on yield and quality of the roots showed that CBSD can decrease root weight in the most susceptible cassava cultivars by up to 70% in Tanzania (Hillocks *et al*., 2001). CMD however, constitutes the most formidable threat to cassava production and can be observed wherever cassava is grown in Africa (reviewed by Legg and Fauquet, 2004). Other production constraints include agronomic problems; soil fertility, water limitation, land degradation, shortage of planting materials, access to markets and limited processing options (COSCA Tanzania, 1996).

2.2. CASSAVA MOSAIC GEMINIVIRUSES

2.2.1 Introduction

Cassava mosaic geminiviruses (CMGs) (Family *Geminiviridae*: Genus *Begomovirus*) are the causal agents of cassava mosaic disease (CMD), which constitutes one of the most widespread and devastating diseases of cassava in Africa (Bock and Woods, 1983; Swanson and Harrison, 1994; Thresh *et al*., 1998). The viral etiology of CMD was first proposed by Storey in 1936, who demonstrated in Tanzania that the disease was graft transmissible from cassava to cassava and inferred that a virus was responsible. No virus was detected until 1975, following isolation and visualization by electron microscopy of geminivirus particles and successful mechanical transmission of sap from cassava to the experimental herbaceous host *Nicotiana benthamiana* and back to a susceptible Brazilian cassava cultivar (Bock, 1975; Bock *et al*., 1981; Bock and Woods, 1983). However, there was initial uncertainty as to the role of the geminivirus that was isolated and characterized, from cassava and it was at first referred to as cassava latent virus (CLV). The situation changed when the virus was shown to cause CMD when transmitted mechanically from herbaceous plants to cassava (Bock and Woods, 1983). The virus was then renamed as *African cassava mosaic virus* (ACMV).

Research progress on CMGs since 1975 has been rapid and much information has been obtained on ACMV structure and composition (Thresh *et al.,* 1994). By 1976, polyclonal antiserum against ACMV had been produced (Bock and Guthrie, 1976) and the virus was shown to contain DNA in 1977 (Harrison *et al.,* 1977). Stanley and Gay (1983) demonstrated the bipartite genome nature of ACMV for the first time and determined its nucleotide sequence. In 1986, monoclonal antibodies against ACMV were produced (Thomas *et al*., 1986) and by 1987, "East'' and ''West'' strains of ACMV were distinguished serologically (Harrison *et al.,* 1987). By 1993 three distinct CMGs had been distinguished serologically namely, *African cassava mosaic virus* (ACMV), *East African mosaic virus* (EAMV) and *Indian cassava mosaic virus* (ICMV) (Hong *et al.,* 1993).

2.2.2 Cassava mosaic disease in Tanzania

Cassava mosaic virus disease (CMD) was first described in Tanzania under the name *Krauselkrankheit* by Warburg (1894). The disease was not reported to cause serious losses until the 1920s (Storey, 1936). Since the 1920s, CMD has been prevalent in the country due largely to the continuous cultivation of susceptible cultivars (Jennings, 1994; Ogbe *et al*., 1997; Legg and Raya, 1998) and the indiscriminate use of diseased propagation material (Storey and Nichols, 1938).

Between 1989 and 1999, CMD incidence in Tanzania was 37% (Thresh *et al.,* 1994). Extensive surveys of CMD conducted in 1993-94 on the Tanzanian mainland and the islands of Zanzibar and Pemba showed disease incidence of 28% on average (Legg and Raya, 1998). CMD incidence was greater along the coastal belt than in the lake zone. The highest disease incidence in the mainland (64.2%) was observed in Tanga and incidence was only 0.7% for the inland Tabora area (Legg and Raya, 1998). In Zanzibar, a recent survey showed that the overall CMD incidence in fields was 71% (Thresh and Mbwana, 1998). Higher CMD incidences (58-90%) have been recorded in southern Tanzania in the highlands along the Lake Malawi shores and varied with crop age. The spread of the severe

CMD pandemic in the Lake Victoria Zone of Tanzania from Uganda has resulted in higher disease incidences of up to 100%, unusually high populations of *Bemisia tabaci* (Genn.) and severe CMD symptoms (Legg and Ogwal, 1998; Legg, 1999). In the CMD- affected areas in the Lake Victoria basin, farmers literally abandoned the crop leading to wide food shortages as was recorded in Uganda in the CMD pandemic-affected areas (Legg, 1999; Ndunguru *et al*., 2003). The characteristics and the coverage of the CMD pandemic in Africa have been recently reviewed by Legg and Fauquet (2004).

2.2.3 Cassava mosaic geminivirus species

Currently six distinct CMG species have been found to infect cassava in Africa: *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) *East African cassava mosaic Zanzibar virus* (EACMZV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Malawi virus* (EACMMV) and *South African cassava mosaic virus* (SACMV)(Fauquet and Stanley, 2003). Further more, two CMG species have been reported from the Indian sub-continent namely *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV) (Hong *et al*., 1993; Swanson and Harrison, 1994; Fauquet *et al*., 2003). CMGs species are distinguished by the nucleotide sequences of their genomic DNA molecules (Hong *et al.,* 1993; Stanley and Gay, 1983; Zhou *et al*., 1997) and by their reactions with a panel of monoclonal antibodies (Harrison and Robinson, 1988; Swanson and Harrison, 1994). On the basis of sequence comparison, whitefly-transmitted geminiviruses have been differentiated into Old and New World (Padidam *et al*., 1995).

2.2.4 Geographical distribution

Until recently, it was considered that CMGs had distinct, largely non-overlapping geographical distribution: ACMV was thought to occur in West, Central and central-southern Africa and EACMV to be restricted to the East African coast, Madagascar,

Malawi and Zimbabwe (Swanson and Harrison, 1994). Recent surveys, however, have shown that EACMV occurs over a much wider area, including western Kenya, western Tanzania, north-eastern Zambia, Nigeria, Togo (Ogbe *et al*., 1996, 1997; Legg and Okao-Okuja, 1999) and most recently in Cameroon and Uganda (Fondong *et al*., 2000; Pita *et al*., 2001a). Recently, EACMV has been reported in the Ivory Coast indicating that the virus could be widely spread in West Africa (Pita *et al*., 2001b). EACMV-UG2 identified as the dominant virus in the CMD-pandemic affected areas, has been reported from the eastern part of the Democratic Republic of Congo (DRC), Burundi, Rwanda central and northern areas of the Republic of Congo (ROC), and eastern Gabon (Legg *et al*., 2001; Neuenshwander *et al*., 2002; Bigirimana *et al*., 2003; Legg *et al.*, 2003, 2004) Therefore the distribution of EACMV isolates is not only limited to East Africa, as previously thought, but includes the rest of West and Central Africa (Fondong *et al*., 2000). ICMV has not been reported in Africa (Swanson and Harrison, 1994) but together with SLCMV occur in India and Sri Lanka (Saunders *et al*., 2002; Fauquet *et al*., 2003).

2.2.5 Impact of cassava mosaic geminiviruses on yield

Whitefly-transmitted geminiviruses cause significant, and often total yield losses of important food and industrial crops in tropical and subtropical agro-ecosystems around the world (Morales and Anderson, 2001). CMD causes severe losses in some areas and achievable yields in Africa are estimated to be decreased by 15 to 24%, which is equivalent to 12 to 13 million tonnes per annum (Thresh *et al.,* 1997). In Uganda, the severe CMD epidemic caused drastic decreases in cassava planting from 26.000 ha in 1989 to only 3000 ha in 1992 in some districts suggesting that the disease had caused the a sharp decline in cassava production (Otim-Nape *et al*., 2001). The impact was greatest in the north-eastern districts of Soroti and Kumi, in which there was a heavy reliance on the cassava cultivar *Ebwanatereka* that proved to be highly sensitive to the CMD and cassava production in the two districts declined by 80-90% between 1990-1993 (Thresh *et al*., 1994). Yield losses are higher when CMG-infected cuttings are used as planting material (Fauquet *et al*., 1988)

than when plants are infected later by the whitefly. Studies conducted in Kenya by Seif (1982) revealed cassava yield reduction of between 24 to 75% under field conditions and there was a strong correlation between CMD severity and yield loss. As intensity of symptoms increased, yield declined dramatically (Fauquet and Fargette, 1990). In Tanzania, Tidbury (1937) showed significant yield reduction of 76% in wholly CMD-affected plants in Zanzibar. Jennings (1970) reported yield losses of up to 90% in susceptible cassava varieties. Between 1981 and 1989, Msabaha *et al*. (1988) reported contrasting yield losses of between five and 26% in late-planted cassava in southern Tanzania and 80 to 90% elsewhere in the country. Recently the pandemic of severe CMD that has spread into Tanzania from Uganda has caused significant yield losses in Kagera region (north western Tanzania) and a common response by farmers was the complete abandonment of cassava production (Legg, 1999). Most recently, Legg and Thresh (2003) used country-level incidence figures obtained from recent surveys carried out in all the major cassava producer countries, together with the 30-40% yield loss assumption to estimate that continental losses in 2003 ranged from 19-27 million tonnes (FAO, 2003). For more information on the yield losses caused by CMD in Africa see Legg and Fauquet (2004).

2.2.6 Host ranges

CMGs infect seven *Manihot* species and a closely related euphorbiaceous species, *Jatropha multifida* L. (Bock *et al*., 1978). Two other species, *Hewittia sublobata* (L.f) Kuntze (Convolvulaceae) and *Laportea aestuans* (L.) Chew (Urticaceace), are suspected to be natural hosts for ACMV in Kenya and West Africa, but the virus has not been transmitted from them back to cassava (Fauquet and Fargette, 1990). Although the host range of CMGs is narrow (Bock *et al*., 1978), they can infect *Nicotiana* spp and *Datura stramonium* L. when they are mechanically inoculated. They produce symptoms typical of CMGs (Briddon *et al.,* 1990).

2.2.7 Transmission and spread

Cassava mosaic geminiviruses (CMGs) are transmitted in a persistent manner by the whitefly, (*Bemisia tabaci* (Gennadius.) (*Homoptera*: Aleyrodidae) (Chant, 958; Dubern, 1979, 1994). The first demonstration that cassava mosaic disease (CMD) is transmitted by a whitefly of the genus *Bemisia* was made in what is now the Democratic Republic of Congo (DRC) by Kufferath and Ghesquiére (1932). Subsequent studies showed that *B. tabaci* is the sole vector and have refined the understanding of the transmission process through the qualification of the optimum acquisition, latent transmission and retention period (Durben, 1979, 1994). The nature of transmission was proven to be transtadial but not transovarial (Dubern, 1994), with minimum times for acquisition, latent period, and inoculation of 3.5 hrs, 3.5 hrs and 5-10 minutes, respectively. Transmission efficiency has varied from very low (0.15-1.7%) for field-collected insects (Fargette *et al*., 1985) to moderate (4-13%) for laboratory-reared insects (Durbern, 1994; Maruthi *et al*., 2002). Recent studies have suggested that there is only limited co-adaptation between virus and vector within Africa, as the frequencies of transmission of different CMGs by *B. tabaci* populations from geographically distant locations in Africa were not significantly different (Maruthi *et al*., 2002). The viral coat protein plays a predominant role in virus transmission (Roberts *et al*., 1984; Briddon *et al*., 1990). Specificity of whitefly-transmitted geminiviruses (WTGs) probably resides at the haemocoel/salivary gland barrier since a non-transmitting species of whitefly, *Trialeurodes vaporariorum* Westwood, can acquire ACMV in the haemocoel but not transmit it (Briddon *et al*., 1990). The rapidity with which CMGs are spread by the whitefly depends on the susceptibility/resistance of the varieties grown, sensitivities of the varieties grown, inoculum or infection pressure, phytosanitation measures and the extent to which CMGs are systemic within the infected plants (Fargette *et al*., 1994). Whitefly mobility is a key factor in the epidemiology of CMD. The higher incidence of severe CMD in the pandemic in eastern and central Africa is usually associated with high *B. tabaci* infestation in cassava (Legg, 1999; Ndunguru *et al*., 2003).

2.2.7 Symptom expression

CMD symptoms described by Storey (1936) included a well-marked mosaic pattern with pale chlorotic areas on leaves, severe stunting and leaf distortion. Less severe symptoms consisted of ill-defined mosaic patterns, green mosaic with slight or absent leaf distortion. Symptoms incited by CMGs vary from mild to severe depending on virus strain, isolate, species, cassava cultivars and environmental factors. In general, cassava plants expressing mild symptoms develop normally with leaves showing mild, light-green mosaic symptoms (Pita *et al*., 2001a; Fauquet and Fargette, 1990). Plants expressing severe symptoms display extreme shrinking of leaves, along with distortion at the bases of the leaflets and distinct chlorosis as is in the case of the severe strain (EACMV-UG2) or those caused by mixed infection of EACMV-UG and EACMV-UG2 (Pita *et al.,* 2001a). Irrespective of the strains, there is no clear distinction in symptoms produced by ACMV and EACMV. However, mixed infection of the two has resulted in severe symptoms (Fondong *et al*., 2000; Pita *et al*., 2001b).

The most visible symptom of CMD is the expression of a characteristic leaf mosaic, and young plants are more severely affected than old ones. Symptoms range from barely perceptible mosaic to stunting of plants and extreme reduction of the leaf blades (Fauquet and Fargette, 1990). However, variations in symptom expression and severity within the same cassava variety have been observed in Cameroon by Fondong *et al*. (1998) and in Uganda by Pita *et al*. (2001a).

Studies conducted by Pascal *et al*. (1993) demonstrated that expression of BL1 gene (homologue of BC1) was responsible for the disease symptoms suggesting that BL1 may interfere with cell-to-cell movement in the vascular system. *Nicotiana benthamiana* L. plants inoculated with plasmid carrying BC1, and BV1 and BC1+BV1 genes from *African cassava mosaic virus* (ACMV) showed symptoms only when both BC1 and BV1 were present (von Arnim *et al*., 1993) confirming the role of DNA-B in efficient virus spread and symptom induction in *N. benthamiana* (Stanley, 1983). As a result of the foliar

symptoms, the ability of the plant to synthesize food is reduced and there is no root bulking (Thresh *et al*., 1994).

2.2.9 Genomic organization

Cassava mosaic geminiviruses have a genome comprising two circular single-stranded (ss) DNAs (A and B) (Stanley and Gay, 1983; Harrison, 1985). The geminivirus DNAs are encapsidated in twinned (geminate) particles (Bock *et al*., 1978). The size of DNA-A and DNA-B is about 2.7-2.8 kbp each. DNA-A contains six genes or open reading frames (ORF) distributed between the virion (V) and complementary (C) sense strand of a double-stranded (ds) DNA intermediate (Stanley *et al*., 1986; Hong and Stanley, 1995). These genes are namely AC1, AC2, AC3 and AC4 on the complementary-sense strand and AV1 and AV2 on the virion-sense (Etessami *et al*., 1991; Morris *et al.,* 1991; Townsend *et el*., 1985). DNA-B encodes two genes BC1 on the complementary-sense strand and BV1 on the virion-sense (Etessami *et al*., 1988) (Fig. 2.1). The two DNA components share only a common region (CR) of approximately 200 bp with high sequence identity of between 90 and 100% (Pita *et al*., 2001a). The CR contains promoter and sequence elements required for DNA replication and transcription (Zhan *et al*., 1991; Eagle *et al*., 1994; Laufs *et al*., 1995a; Chatterji *et al*., 1999). The common region is located in the intergenic region on both DNA components (Revington *et al*., 1989; Lazarowitz *et al*., 1992)

The CR shows one potentially very stable hairpin structure between nucleotides 133 and 165, and contains a GC-rich inverted repeat that could form a stem loop structure, the loop of which is composed almost entirely of A and T residues (Stanley and Gay, 1983). An invariant AT-rich sequence 5′-TAATATTAC in the loop is found in all geminivirus genomes (Revington *et al*., 1989; Lazarowitz *et al*., 1992).

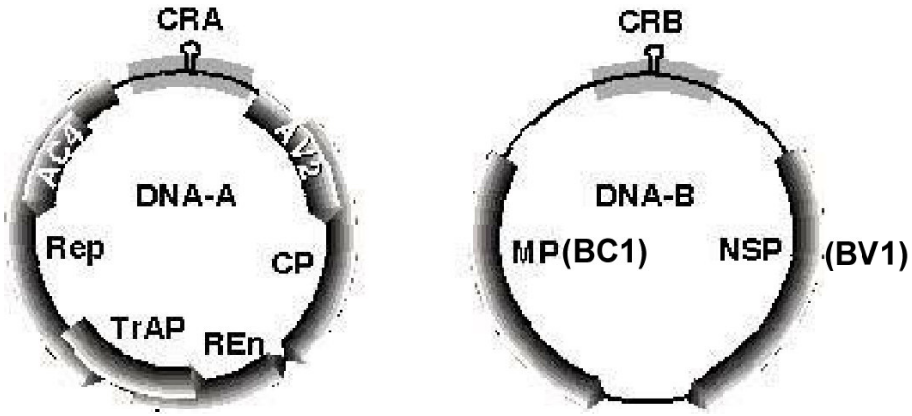

Fig. 2.1. Bipartite genome organization of cassava mosaic geminiviruses. The part of intergenic region whose sequence is identical in both begomovirus components is called the common region (CR) and includes a sequence capable of forming a stable hairpin loop structure. CP, coat protein; MP, movement protein; Rep, replication initiator protein; TrAP, *tr*anscription *a*ctivator *p*rotein; Ren, *r*eplication *e*nhancer *p*rotein. The proteins encoded by the BV1 and BC1 ORFs are both movement proteins. MP, movement protein; NSP, *n*uclear *s*huttle *p*rotein.

2.2.10 Cassava mosaic geminivirus gene products and their role in pathogenicity

Both DNA-A and DNA-B of CMGs are required to establish infection in plants. DNA-A encodes all viral functions necessary for replication and encapsidation of both viral DNAs, while DNA-B encodes functions necessary for movement of the virus DNA through the infected plant (Rogers *et al*., 1986; Sunter *et al*., 1987; Unseld *et al*., 2004).

The gene products are named according to genome component (A or B) (Sunter *et al*., 1990). The products of ORFs on the DNA-A genome components are Rep/AC1 (Replication-associated protein), TrAP/AC2 (transcription activating protein), REn/AC3 (replication enhancer) and AC4. The ORFs encoding Rep, TrAP, and REn partially overlap, and a small ORF, AC4, is located within the Rep ORF, but in a different reading frame (Hanley-Bowdoin *et al*., 1999). The gene products of the ORFs AV1 and AV2 are CP (coat protein) and AV2 respectively. The DNA-B component encodes two ORFs, BC1 and BV1 (Haley *et al*., 1992) whose products are the MP (movement protein) and the nuclear shuttle protein (NSP) respectively. The arrangement of the ORFs shows that they are expressed in a bi-directional manner.

Recent advances have identified genes involved in replication, spread of virus or DNA in the plant and insect transmission. CMGs DNA-A can replicate autonomously in protoplasts to produce the dsDNA (replicative intermediate) and ssDNA (genomic) forms typically associated with wild-type infection (Davies and Stanley, 1989; Saunders *et al.,* 1991; Gutierrez, 2000). These data indicate that DNA-A contains all the virus- encoded genes responsible for replication.

Mutational analysis has indicated that the complementary sense gene AC1 is essential and sufficient for DNA replication (Saunders *et al.,* 1991; Kong *et al*., 2000). Two of these proteins, Rep and REn are required for efficient viral DNA replication. The Rep is essential for replication, whereas REn enhances viral DNA-Accumulation by an unknown mechanism. Rep/AC1 initiates and terminates virus DNA plus strand replication (Laufs *et al*., 1995a; Orozco *et al*., 1996; Zhang and Gruissem, 2003) and specifically binds to

double stranded DNA (dsDNA) during origin recognition (Fontes *et al*., 1994; Castellano *et al*., 1999). Rep can also hydrolyse ATP and interact with itself (Orozco *et al*., 1996) and with the viral replication enhancer REn (Settlage *et al*., 1996) as well as other plant host factors (Zhang and Gruissem, 2003; Castillo *et al*., 2004); Hanley-Bowdoin *et al*., 2004). The Rep binds to common region sequences upstream of the conserved nonanucleotide motif TAATATTAC (Fontes *et al*., 1994) implicated in the initiation of the rolling cycle replication (Saunders *et al*., 1991; Stanley, 1995). It has been demonstrated that Rep binds specifically to a 13 bp element (GGTAGTAAGGTAG) located on the left side of the intergenic region between the transcription start point and the TATA box of the Rep promoter and initiates replication by introduction of a nick in the plus-strand of the conserved nonanucleotide sequence (TAATATT↓AC) (Laufs *et al*., 1995b; Stanley, 1995).

A function is yet to be assigned to the overlapping gene AV2 (Hong and Stanley, 1995). AC2 encoded product (TrAP) *trans*-activates the expression of the coat protein (*CP)* gene AV1 and DNA-B gene BV1 (movement protein gene) (Sunter and Bisaro, 1992). The activation occurs at the level of transcription (Sunter and Bissaro, 1992) whereby it is reported to activate the AV1 and BV1 promoters (Jeffrey *et al*., 1996). A mutagenesis of the AC3 open reading frame of African cassava mosaic virus DNA-A reduces DNA-B replication and ameliorates disease symptoms (Morris *et al*., 1991). This further qualifies the name of AC3 protein as Replication Enhancer; REn. No function has been assigned to AC4 in CMGs, although homologues in *tomato leaf curl virus* (TLCV) and *beet curl top virus* (BCTV), have been implicated in either symptom development or virus movement (Stanley and Latham, 1992; Jupin *et al.,* 1994; Ridgeman *et al.,* 1994).

Virion-sense gene AV1 product (CP) is a multifunctional protein. It determines the vector specificity (Briddon *et al*., 1990) and protects viral DNA during transmission by the insect vector (Azzam *et al*., 1994) or mechanical transmission (Frischmuth and Stanley, 1998). Although bipartite geminiviruses do not require the CP for systemic infection of some plant species (Stanley and Townsend, 1986; Gardiner *et al*., 1988), symptoms are often attenuated and the onset of symptoms is delayed when plants are systemically infected with CP mutants (Etessami *et al*., 1989). Apart from the involvement of CP in viral

infection, particularly on particle formation (Unseld *et al*., 2004), using coat protein transient expression experiments, putative sequence motifs of ACMV CP involved in nuclear import and export have been identified (Unseld *et al*., 2001). There is increasing evidence that CP plays a role in plant-plant spread of CMGs by *B. tabaci.* This is because there is a high amino acid homology between geminiviruses transmitted by similar or identical whitefly, suggesting that the specificity of the transmitting insect may be defined by the coat protein (Davies and Stanley, 1989). Indeed the coat protein is the only viral determinant of whitefly-mediated transmission to be identified to date (Briddon *et al*., 1990) and is the most highly conserved among begomoviral-encoded proteins (Padidam *et al*., 1995).

The two genes located on the DNA-B (BV1 and BC1) have been implicated in cell- to-cell (short distance) and long-distance virus movement (Townsend *et al*., 1985; Etessami *et al*., 1988; von Arnim *et al*., 1993) regulatory. The nuclear shuttling, that is binding of ssDNA-A and DNA-B and exporting them across the nuclear envelope to the cytoplasm, is done by BV1 protein (NSP). It has been noted that BV1 has high arginine content reminiscent of DNA-binding proteins such as protamines and histones (von Arnim *et al*., 1993). The BC1 gene encodes for a movement protein (MP) that, is involved in cell-to-cell movement of virus DNA, symptom development and host range determination (Noueiry *et al.,* 1994; Pascal *et al*., 1994; Sanderfoot *et al*., 1996; Ward *et al.,* 1997).

Analysis of geminivirus movement proteins (MPs) in the context of viral infection has further suggested that BL (homology of BC1) in squash leaf curl geminivirus (SqLCV) and tomato golden mosaic virus (TGMV) influences the symptom phenotype (Pascal *et al*., 1993). In transgenic tobacco plants, expression of BC1 gene is sufficient to produce disease-like symptom (leaf curl and mosaic) typical of the wild-type SLCV (Pascal *et al*., 1993).

2.2.11 Cassava mosaic geminiviruses replication

Geminiviruses do not encode their own DNA polymerases and instead rely on the nuclear DNA replication machinery of the host (Gutierrez, 2000). They replicate their genomes in nuclei of mature cells, which are not competent for replication, so an early step in the geminivirus infection may the induction of host DNA replication enzymes (Hanley-Bowdoin *et al*., 1999; Gutierrez, 2000). CMGs rely on their plant hosts for all of the other enzymes and factors required for replication. CMGs replicate their circular genomes through double-stranded intermediates in the plant nucleus using a rolling circle mechanism (Saunders *et al*., 1991; Stenger *et al*., 1991; Stanley, 1995). Geminivirus DNA replication follows a rolling circle strategy (Saunders *et al*., 1991; Stenger *et al*., 1991), which resembles that of prokaryotic ssDNA replicons (Novick, 1998).

The replication occurs in two stages. The initial stage encompasses the conversion of the ssDNA genome into dsDNA intermediate product (Fig. 2.2; Kammann *et al*., 1991; Saunders *et al*., 1992). This step, poorly understood in molecular terms, must be carried out entirely by cellular enzymes. Initiation of DNA replication during the second stage, the rolling circle phase, requires the concerted action of the viral Rep protein (and perhaps other viral proteins) with cellular factors, and leads to the production of dsDNA-And ssDNA viral forms (Stenger *et al*., 1991; Heyraud *et al*., 1993a; Stanley, 1995). Rep initiates viral DNA replication by binding specifically to reiterates sequence motifs (iterons) within the intergenic region and introducing a nick into the nonanucleotide sequence TAATATT↓AC, universally conserved in all geminiviruses (Fontes *et al*., 1994). Furthermore, Rep performs strand transfer reaction at the viral origin of replication. It binds to the 5′ end of the cleaved strand DNA using its tyrosine and at the conclusion of each round of rolling circle replication, acts as a terminase, releasing a unit-length viral single strand genome (Laufs *et al*., 1995b). After the viral strand of the replicative form (RF) is nicked to prime the plus-strand DNA synthesis, a new viral strand can then be synthesized displacing the original viral strand. The displaced viral strands then serve again as templates for complimentary (minus-strands) synthesis or are packaged into a virion, depending on the stage of the infection (reviewed by Chansan, 1995). The REn

protein, though not essential, will boost viral DNA replication several folds (Sunter *et al*., 1990).

After replication initiation, recruitment of cellular replication factors is a necessary step to complete viral DNA replication. The absolute requirement for host cell DNA replication factors has forced geminiviruses to develop a number of complex interactions with the host cell. Recent experiments have revealed interactions between the Rep protein and cellular DNA replication proteins (Hanley-Bowdoin *et al*., 2004). Rep interacts with several host proteins, including the cell cycle regulators, retinoblastoma protein (Rb) to regulate cell-cycle progression, components of the cell DNA replication such as proliferating cell nuclear antigen (PCNA) to support viral DNA replication, and plant sumoylation system (Kong *et al*., 2000; Kong and Hanley-Bowdoin, 2002; Castillo *et al*., 2004).

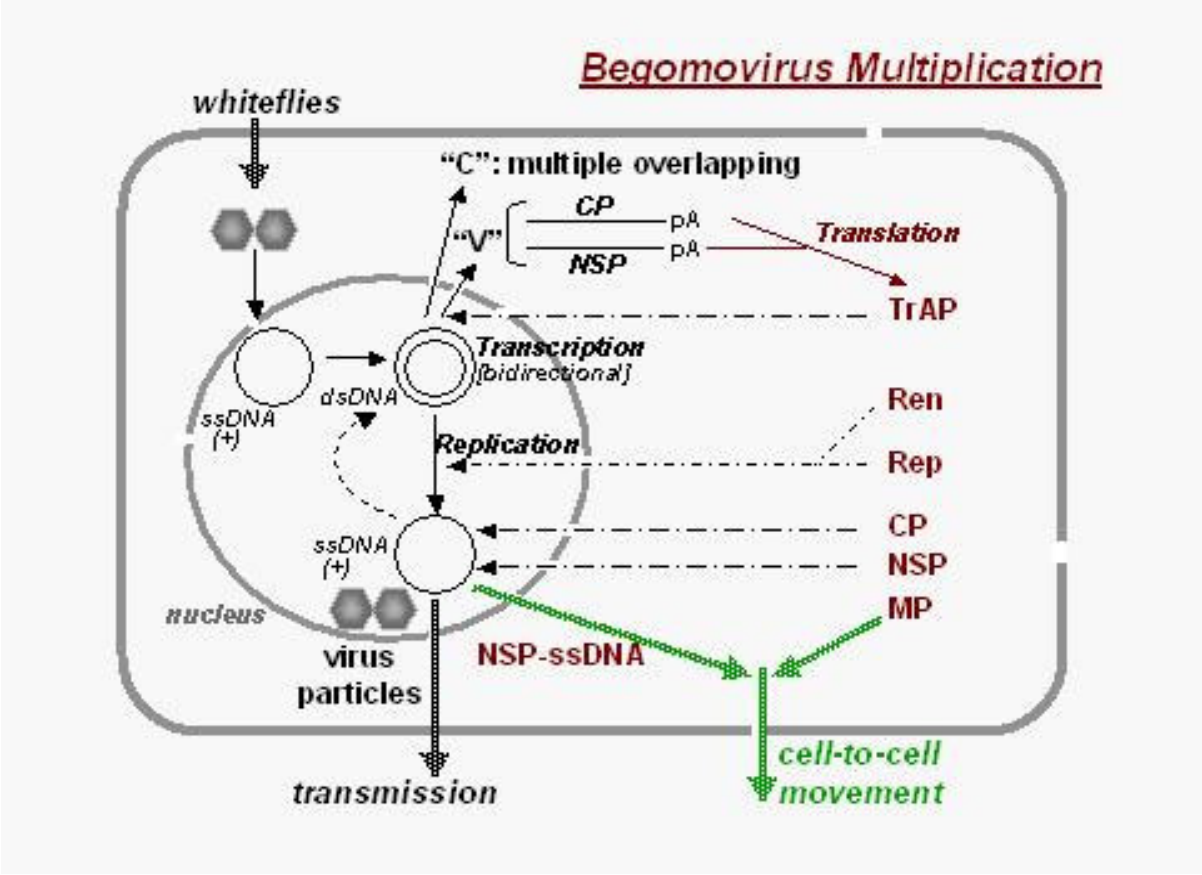

Fig. 2.2. Begomovirus replication in the nucleus of the plant cell. On the infection of plant and following uncoating of genomic component, the encapsidated ssDNA is converted into transcriptionally active dsDNA replicative form prior to gene expression by the synthesis of complementary-sense DNA. The RF dsDNA serve as a template for virus-sense strand synthesis to generate free ssDNA, which then (i) re-enter the DNA replication pool, (ii) associate with CP or (iii) be transported outside the nucleus and to the neighbouring cell, most probably through plasmodesmata, with the help of viral MPs.

2.2.12 Virus movement within the host plants

Virus movement through infected plants can be conveniently divided into phases of local cell-to-cell movement, which is thought to occur through virus-modified plasmodesmata, and long distance systemic movement, which is generally thought to occur through the phloem sieve elements (Noueiry *et al*., 1994). Geminiviruses, which replicate in the nucleus, must also have a mechanism to move intracellularly across the nuclear membrane.

CMGs are able to move systematically in plants as ssDNA or dsDNA-Alone or complexed with viral protein and the movement is mediated by virus-encoded proteins (Maule, 1991). The two essential genes (BV1 and BC1) located on DNA-B of CMGs have been implicated in cell-to-cell and long-distance virus movement (Townsend *et al*., 1986; Etessami *et al*., 1988; von Armin *et al*., 1993; Carrington *et al*., 1996). At the one and two cell level, the cell-to-cell movement pathway involves the transport of newly synthesized genomes to and through plasmodesmata (channels that transverse cell walls and provide cytoplasmic continuity between adjacent cells) and can be thought of as a process of genome movement (Carrington *et al*., 1996). For geminiviruses, the BV1 gene product (NSP) functions as a nuclear shuttle to escort newly synthesized viral DNAs to the cytoplasm through nuclear pores (Carrington *et al*., 1996; Sanderfoot *et al*., 1996). In the cytoplasm, BC1 functions to traffic viral DNAs to and through plasmodesmata. Through plasmodesmata the viruses spread from cell to cell and after crossing the boundary between non-vascular and vascular tissues, they move to other parts of the plant, resulting in systemic infection and development of viral disease (Carrington *et al*., 1996).

2.2.13 Recombination, pseudorecombination and its role in viral pathogenicity

Recombination, defined here as the exchange of genetic information between two nucleotide sequences, is an important process that influences biological evolution at many different levels (Posada and Crandall, 2001). Recombination explains a considerable amount of genetic diversity and in general, is considered to be one of the driving forces for

virus evolution and cassava mosaic geminiviruses provide prime examples of this phenomenon with recombination occurring in both DNA-A and DNA-B (Padidam *et al*., 1999; Pita *et al*., 2001b). Mixed infection occurs frequently in cassava plants in nature (Ogbe *et al*., 1996; Harrison *et al*., 1997; Legg and Okau-Okuja, 1999; Fondong *et al*., 2000; Pita *et al*., 2001a) and provides the opportunity for recombination to occur between viruses. Sequence comparison of a large number of geminiviruses species and strains has shown that recombination is a very common occurrence, and has an important role to play in the evolution of these viruses (Padidam *et al*., 1999).

For CMGs, an important distinction can be drawn between ACMV, which shows a high degree of homology regardless of location of collection, and EACMV-like viruses, for which recombination is frequent and variation is considerable (Pita *et al*., 2001a). A severe form of CMD that appeared in Uganda in the late 1980's is apparently associated with the appearance of a new geminivirus (EACMV-UG2), the genomic DNA-A of which has arisen by recombination between DNA-A of ACMV and of EACMV (Deng *et al*., 1997; Zhou *et al*., 1997; Pita *et al*., 2001a). Most of the coat protein gene of EACMV-UG2 is derived from ACMV, from which EACMV-UG2 was serologically indistinguishable, but the 5′ and 3′ parts of the gene, and the remainder of DNA-A are derived from EACMV (Deng *et al*., 1997; Zhou *et al*., 1997). Another recombination has been reported in Cameroon between EACMV-[CM]/IC and EACMV-[CM] with the recombined fragment in the AC2-AC3 region of DNA-A and the BC1 region of DNA-B (Fondong *et al*., 2000). Complete nucleotide sequences of DNA-A of SACMV analysis revealed one significant recombination event spanning the entire AC4 ORF (Berrie *et al*., 1998). Furthermore, comparison of DNA-A among isolates of EACMV from Kenya, Malawi and Tanzania has revealed intergenic region sequence dissimilarities between types 1 (EACMV-TZ) and type 2 (EACMMV) and it has been suggested that recombination has played a role in their divergence and that *ori* may be a recombination point (Zhou *et al*., 1998). In this case recombined portions of the AV2 in EACMMV showed strong sequence identity with a strain of tomato yellow leaf curl virus (Zhou *et al*., 1998).

The exchange of the entire genomic components (pseudorecombination) between closely related isolates (Stanley *et al*., 1986) and distinct species (Hou and Gilbertson, 1996) may also contribute to geminivirus diversity. Pita *et al.* (2001a) found a natural pseudorecombinant virus EACMV-UG2 DNA-A+ EACMV-UG3 DNA-B in Uganda. This pseudorecombinant that was infectious to biollistically inoculated cassava plants was the most frequently occurring form found in the field and could be a key to the CMD epidemic found in Uganda. EACMV-UG3 DNA-A is an as-yet undescribed geminivirus found in cassava samples collected in Uganda. An artificial pseudorecombinant between ACMV-Nigerian isolate designated ACMV-NOg and ACMV isolate from Kenya (designated ACMV-KE) has been reported (Briddon *et al*., 1998). ACMV-NOg (DNA-A) and ACMV-KE (DNA-B) was not infectious to cassava while ACMV-KE DNA-A and ACMV-NOg DNA-B was infectious producing typical mosaic in cassava plants. The progeny of this pseudorecombination are transmissible by *B. tabaci* and infectious to cassava by biolistic inoculation (Briddon *et al*., 1998). Recombination and pseudorecombination has been reported to occur in other geminiviruses. For example, recombination that occurred among begomoviruses infecting cotton in Pakistan resulted into an epidemic of cotton leaf curl disease (Zhou *et al*., 1998). Pseudorecombination between tomato mottle virus (ToMoV) and bean dwarf mosaic virus (BDMV) resulted into an increased pathogenicity when viable ToMoV DNA-A plus BDMV DNA-B pseudorecombinant was maintained in *N. benthamiana* and *Phaseolus vulgaris* (Hou and Gilbertson, 1996). Mixed infections are thought to be one of the prerequisites for development of new recombinant virus or strains (reviewed by Gallitelli and Accotto, 2001). Mixed infections of ACMV and EACMV in the field have been reported (Fondong *et al*., 2000; Pita *et al*., 2001, Ogbe *et al*., 2003). It is apparent that interspecies recombination is very frequent and generates variability allowing the emergence of new strains and new species of gemminivirus.

2.2.14 Molecular identification and characterisation

Because of the increasing importance of CMGs, rapid accurate methods are needed for virus detection and subsequent identification. Such methods would greatly facilitate studies of the epidemiology and the genetic diversity of these viruses. DNA-based diagnostic approaches, including polymerase chain reaction (PCR) amplification and DNA sequencing, are useful in CMGs identification and characterization (Padidam *et al*., 1995). Southern hybridization analysis of PCR-amplified fragments with cloned CMGs DNA components as probes can be useful in CMGs characterisation (Rojas *et al*., 1993; Brown *et al*., 2001; Pita *et al*., 2001a). Through restriction fragment length polymophism (RFLP) analysis of PCR-amplified fragments by digesting the fragments with one or more endonucleases, known CMGs have been distinguished from uncharacterized geminiviruses (Rojas *et al*., 1993).

Using sequence comparison analysis of complete nucleotides of DNA-A and DNA-B, coat protein gene, and DNA-B genes, it is possible to differentiate CMGs into isolates, species and strains (Padidam *et al*., 1995). The criteria utilized to distinguish between CMGs are 90-100% for isolates, 80-90% for strains and at 89% for species demarcation for DNA-A component (Fauquet and Stanley, 2003).

The ability to combine the development of 'core' region CP primers that exclusively target only the begomovirus CP with existing PCR technology and automated sequencing permit both rapid detection of whitefly-transmitted geminiviruses and, ultimately, virus identification based on virus nucleotide sequences. Sequencing of the core and entire coat protein and comparison of the deduced amino acids of a South African isolate of CMGs (SACMV) revealed amino acid differences of less than 90% when compared to other WTGs (Berrie *et al*., 1997, 2001). These differences were considered great enough to justify SACMV as a distinct virus from ACMV, EACMV, and TYLCV. Comparison of CP nucleotide sequences has shown the EACMV isolate from Cameroon as being different from those previously reported in East Africa (Uganda, Kenya and Tanzania) (Fondong *et al*., 2000). Analysis of CP and complete nucleotide sequences of DNA-A and DNA-B of

various CMG isolates in Uganda has confirmed the occurrence of different strains for the two areas namely ACMV-UG, EACMV-UG1, EACMV-UG2 (formerly EACMV-UG/UGV) and EACMV-UG3 (Deng *et al*., 1997; Zhou *et al*., 1997; Pita *et al*., 2001a). The molecular techniques used for identification of CMGs have been improved to allow for detection of virus recombinants and pseudorecombinants within the begomoviruses as successfully been demonstrated by Pita *et al*. (2001a) and Fondong *et al*. (2000).

2.2.15 Cassava mosaic geminiviruses in East Africa

Prior to 1997, only two CMGs were recognized in East Africa: EACMV and ACMV (Hong *et al.,* 1993). By 1994, CMD in Tanzania was found to be caused by ACMV and EACMV with ACMV occurring largely in the central and western part of the country and EACMV in the coastal region (Swanson and Harrison, 1994).

In a survey conducted in 1996-97 in Tanzania it was found that most samples collected in the coastal areas contained only EACMV. The samples from western Tanzania had both ACMV and EACMV. Both ACMV and EACMV were found in different areas, but ELISA-based diagnostic could not detect mixtures, which were suspected to occur (Obge *at al*., 1996, 1997).

In the second half of 1999, East African cassava mosaic virus from Uganda (EACMV-UG2)(recombinant in the CP) was reported for the first time in Bukoba district, Kagera region, on the border with Uganda (Legg and Okao-Okuja, 1999) and since then has been found in the entire Kagera region (north western Tanzania). The EACMV-UG2 appears to be a recombinant hybrid of EACMV and ACMV (Deng *et al.,* 1997; Zhou *et al*., 1997). The virus was found to associate with the high disease incidence characteristic of the severe CMD pandemic that has been spreading southward from Uganda (Legg, 1999; Legg and Okao-Okuja, 1999; Otim-Nape *et al*., 1997) currently causing serious damage to cassava in Uganda, Kenya, Tanzania, Sudan, D.R Congo and Rwanda (Legg, 1999). Another virus (EACMV-UG3) has been reported in Uganda (Pita *et al.,* 2001a) and it is

frequently found to be associated with EACMV-UG2 (Pita *et al.,* 2001a). Maruthi *et al*. (2002) have reported the occurrence of a distinct cassava mosaic geminivirus in Zanzibar, Tanzania, in samples collected from plants expressing mild disease symptoms based on DNA-A and DNA-B sequence phylogenetic comparison.

2.2.16 Synergism between virus species

Synergism between members of CMGs is a common phenomenon. A synergistic interaction between ACMV and EACMV has been suggested by Harrison *et al*. (1997) and demonstrated by Fondong *et al*. (2000) with infectious clones of ACMV-[CM] and EACMCV isolates from Cameroon. Cassava plants co-infected by ACMV–CM and EACMCV showed unusually severe symptoms under field conditions and in the growth chamber compared with singly infected plants (Fondong *et al*., 2000). This suggested a synergistic interaction between the two viruses. The interaction increases DNA-Accumulation of both viruses and therefore their capacity to be transmitted by whiteflies (Pita *et al.,* 2001a).

Mixed ACMV and EACMV infections were reported to be an important feature of the severe CMD first reported from Uganda and subsequently in neighboring countries (Harrison *et al*., 1997; Legg, 1999; Pita *et al*., 2001a). Plants infected with EACMV-UG2 and ACMV expressed more severe symptoms than both of the single infection and measurement of virus load in the infection conditions suggested occurrence of synergistic interaction between the two viruses (Pita *et al*., 2001a). In the severe CMD epidemic situation, dual infection involving EACMV-UG2 and ACMV is frequently encountered at the epidemic front. Elsewhere in eastern and western Africa, ACMV and EACMV are known to occur together resulting in severe CMD symptoms in the fields (Fondong *et al*., 2000; Pita *et al*., 2001a; Ogbe *et al*., 1997, 2003). Synergism is of primary importance for the emergence of new geminivirus diseases and has been shown to be a key factor in the genesis and spread of the CMD pandemic in East and Central Africa (Harrison *et al*., 1997; Legg, 1999; reviewed by Legg and Fauquet, 2004).

2.2.17 Control strategies

Phytosanitation involves the use of various means of improving the health status of cassava planting material and for decreasing the availability of sources of infection from which further spread of CMGs can occur through the activity of the whitefly vector. Thresh *et al*. (1998) described three main features of phytosanitation for the control of CMD and they include crop hygiene, the use of virus-free stem cuttings as planting material and roguing of diseased plants from within stands. Crop hygiene facilitate the control of CMD by removing the debris and surviving plants of previous crops to decrease the risk of carry-over of inoculum to any new plantings at the site or nearby (Fargette *et al*., 1990). The use of virus-free cuttings as planting materials greatly enhances productivity and decreases the extent of infection in the locality and the opportunity for further spread by vectors. Although little attempt has been made to use virus-free stocks of cassava, this is a basic approach to CMD control and one that has been widely adapted for control of viruses in many vegetatively propagated crops (reviewed by Legg and Fauquet, 2004). Approaches to the provision of virus-free germplasm involving the 'clean-up' of tissue culture material through meristematic tip and thermotherapy have been proposed and to date are largely been confined to quarantine support facility (reviewed by Legg and Fauquet, 2004). Roguing or the removal of CMD-diseased plants from within a crop stand is a well known means of disease control (Thresh, 1988) particularly in the official schemes of multiplication of planting material. However, rouging is often unpopular with producers-since the loss of the plants removed is considered to outweigh the future benefits that may results from reduced virus spread (Fofana *et al*., 2003; Legg and Fauquet, 2004).

Conventional resistance breeding has been used for the control of CMD in Africa and several advances have been made. The earliest breeding program was initiated at Amani station in north-eastern Tanzania in 1930s and used both intra-specific and inter-specific crosses with *Manihot glaziovii* Muell.-Arg. to produce progeny with increased levels of resistance to CMD. In 1950s, the program was terminated but one of the progeny seed

from one of the most restsistant clones, 5318/34, was used to initiate work at IITA (International Institute of Tropical Agriculture) from 1970 (Hahn *et al*., 1980). Some of the most important CMD-resistant cones from the Tropical Manihot Species TMS series that resulted from this work and now widely distributed across the African continent in main cassava producing countries include: TMS 4(2)1425, TMS 30337, TMS 91934, TMS 30001, TMS 60142 and TMS 30572 (reviewed by Legg and Fauquet, 2004). Recently IITA has been exploiting newly identified sources of resistance conferred by a single dominant gene/locus (CDM2), which is derived from a Nigerian landrace (Akano *et al*., 2002). Crosses involving *M. gaziovii* resistance with CMD2 have given rise to progeny, which are nearly immune to CMD. The discovery of single dominant gene/locus conferrering resistance to CMD has recently opened up new opportunities for marker-assisted selection (MAS) (Akano *et al*., 2002).

A new phenomenon called post-transcriptional gene silencing (PSTG) has recently been shown to be responsible for the inherent ability of many plants to specifically degrade nucleic acids in sequence specific manner, including those of viruses (Hamilton and Baulcombe, 1999). This strategy has been successfully employed to engineer virus resistance. A proof that RNAi can be engineered to effectively control geminiviruses has recently been documented in transgenic assays for ACMV (Vanitharani *et al*., 2003). A successful generation of transgenic cassava line (Y85) resistance to ACMV as well as EACMV has been reported. Detection of the transgene-derived siRNAs and the extremely low transgene product (the truncated Rep protein from ACMV) in this line suggests that RNA silencing is the mechanism responsible for the resistance.

Another approach has been the use of anti-sense RNA technology in which targets for the anti-sense interference were the mRNAs of AC1, AC2, and AC3 of ACMV (Zhang *et al*., 2004). Virus accumulation assays in transgenic plants revealed reduced levels or inhibited replication of ACMV. In another novel approach, a hypersensitive response upon infection is elicited through the transformation of a very susceptible genotype, TMS 60444 with the bacteria *barnase* and *barstar* genes from *Bacillus amyloliquefaciens*, controlled by ACMV a binary promoter (Zhang *et al*., 2003). Reductions of viral replication of between 86% and

99% have been demonstrated when comparing leaves of untransformed and transgenic plants in greenhouse experiment.

2.3 REFERENCES

Akano, A.O., Dixon, A. G. O., Mba, C., Barrera, E. & Fregene, M. (2002). Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theoretical Applied Genetics* **105**, 521-535.

Azzam, O., Frazer, J., de la Rosa, D., Beaver, J. S., Ahlquist, P. & Maxwell, D.P. (1994). Whitefly transmission and efficient ssDNA accumulation of bean golden mosaic geminivirus requires functional coat protein. *Virology* **204,** 289-296.

Berrie, L. C., Palmer, K., Rybicki, E. P., Hiyadat, S. H., Maxwell, D. P. & Rey, M. E. C. (1997). A new isolate of African cassava mosaic virus in South Africa. *African Journal of Root and Tuber Crops* **2**, 49-52.

Berrie, L. C., Palmer, K. E., Rybicki, E. P. & Rey, M. E. (1998). Molecular characterization of a distinct South African cassava infecting geminivirus. *Archives of Virology* **143**, 2253-2260.

Berrie, L. C., Rybicki, E. P. & Rey, M. E. C. (2001). Complete nucleotide sequence and host range of South African cassava mosaic virus: Further evidence for recombination amongst begomoviruses. *Journal of General Virology* **82**, 53-58.

Bigirimana, B., Barumbaze, P., Obonyo, R., & Legg, J. P. (2003). First evidence for the spread of *East African cassava mosaic virus*-Uganda (EACMV-UG) and the pandemic of severe cassava mosaic disease to Burundi. *New Disease Report* [http://www.bspp.org.uk/ndr/] Volume 8.

Bock, K, R. (1975). Maize streak, cassava latent and similar viruses. In: Abstracts of the Third International Congress for Virology, Madrid, Spain, p 93.

Bock, K. R. & Guthrie, E. J. (1976). *Recent advances in research on cassava viruses in East Africa. In: African cassava mosaic.* pp. 11-16. Edited by B. L. Nestel. Report of interdisciplinary workshop. Muguga, Kenya. EAFFRO/IDRC

Bock, K. R., Guthrie, E. J. & Meredith, G. (1978). Distribution, host range, properties, and purification of cassava latent virus, a geminivirus. *Annals of Applied Biology* **90**, 360-367.

Bock, K. R., Guthrie, E. J. & Fagueiredo, G. (1981). A strain of cassava latent virus occurring in coastal districts of Kenya. *Annals of Applied Biology* **99**, 151-159.

Bock, K. R. (1983). *Epidemiology of cassava mosaic disease in Kenya. In: Plant virus Epidemiology.* pp. 337-347. Plumb, R. T and J. M. Thresh. Eds, Blackwell Scientific Publications, Oxford, UK.

Bock, K. R. & Woods, R. D. (1983). Etiology of African cassava mosaic disease. *Plant Disease* **67**, 994-995.

Briddon, R. W., Liu, S., Pinner, M. S. & Markham, P.G. (1998). Infectivity of African cassava mosaic virus clones to cassava by biolistic inoculation. *Archives of Virology* **143**, 2487-2492.

Briddon, R.N., Pinner, M. S., Stanley, J. & Markham, P. G. (1990). Geminivirus coat protein replacement alters insect specificity. *Virology* **177**, 85-94.

Brown, K. K., Idris, A. M., Torres-Jerez, I., Banks, G. K. & Wyatt, S. D. (2001).

The core region of the coat protein gene is highly useful for establishing the provisional identification on and classification of begomoviruses. *Archives of Virology* **146**, 1581-1598.

Castellano, M, M., Sanz-Burgos, A. P. & Gutierrez, C. (1999). Initiation of DNA replication in eukaryotic rolling circle replication: Identification of multiple DNA-protein complexes at the geminivirus origin. *Journal of Molecular Biology*, **290,** 639-652.

Castillo, A. G., Kong, L. J., Hanley-Bowdoin, L & Bejarano, E. R. (2004). Interaction between a geminivirus replication protein and the plant sumoylation system. *Journal of Virology* **78,** 2758-2769.

Carrington, J. C., Kasschau, K. D., Mahajan, S. K. & Schaad, M. C. (1996). Cell to cell and long distance transport of viruses in plants. *The Plant Cell* **8**, 1669-1681.

Chant, S. R. (1958). Studies on the transmission of cassava mosaic virus by *Bemisia* spp. (Aleyrodidae). *Annals of Applied Biology* **46,** 210-215.

Chasan, R. (1995). Geminiviruses: A twin approach to replication. *The Plant cell* **7**, 659-660.

Chatterji, A., Padidam, M., Beachy, R. N. & Fauquet, C. M. (1999). Identification of replication specificity determinants in two strains of tomato leaf curl virus New Delhi. *Journal of Virology*, **73**, 5481-5489.

COSCA Tanzania. (1996). *Production Prospects for cassava in Tanzania (draft).* Collaborative Study of Cassava in Africa (COSCA) Working Paper No. 16. International Institute of Tropical Agriculture (IITA) and Ministry of Agriculture, Tanzania.

Davies, J. W. & Stanley, J. (1989). Geminivirus genes and vectors. *Trends in Genetics* **5**, 77-81.

Deng, D., Otim-Nape, W. G., Sangaré, A., Ogwal, S., Beach, R. N. & Fauquet, C. M. (1997). Presence of a new virus closely related to East African cassava mosaic geminivirus, associated with cassava mosaic outbreak in Uganda. *African Journal of Root and Tuber Crops* **2**, 23-28.

De Bruijn, G. H. & Fresco, L. O. (1989). The importance of cassava in world food production. *Netherlands Journal of Agricultural Science* **37**, 21-34.

Dubern, J. (1979). Quelques proprieties de la mosaique Africaine du Manioc: La transmission. *Phytopathology Zeitschrift* **96**, 25-39.

Dubern, J. (1994). Transmission of African cassava mosaic geminivirus by whitefly (*Bemisia tabaci*). *Tropical Science* **34**, 82-91.

Eagle, P. A., Orozco, B. M. & Hanley-Bowdoin, L. (1994). A DNA sequence required for geminivirus replication also mediates transcriptional regulation. *Plant Cell* **6**, 1157-1170.

Etessami, P., Collis, R., Ellwood, S. & Stanley, J. (1988). Delimination of essential genes of cassava latent virus DNA 2. *Nucleic Acid Research* **16**, 4811-4829.

Etessami, P., Watts, J. & Stanle, J. (1989). Size reversion of African cassava mosaic virus coat protein gene deletion mutants during infection of *Nicoatiana benthamiana*. *Journal of general Virology* **70,** 277-289.

Etessami, P., Saunders, K., Watts, J. & Stanle, J. (1991). Mutational analysis of complementary-sense genes of African cassava mosaic DNA-A. *Journal of General Virology* **72**, 1005-1012.

FAO, (1997a). *Draft working paper notes on selected chapters of "The World*

Cassava Economy: Recent trends and medium-term outlook''. Global cassava development strategy; Progress review workshop, International Fund for Agricultural development. Rome, Italy.

FAO. (1997b). FAO Production Year Book **85**, 88.

FAO. (2000). *Championing the cause of cassava*. FAO News and Highlights.

FAO, (2001). FAO Production year book for 2000. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO. (2003). Cassava production data 2002. (http:/www.fao.org).

Fargette, D. (1985). *Epidemiologie de la mosaìque Africaine du manioc en Ivory Coast*. PhD thesis. pp 203. Science and Technology University, Montpellier.

Fargette, D., Fauquet, C., Grenier, E. & Thresh, J. M. (1990). The spread of African cassava mosaic virus into and within cassava fields. *Journal of Phytopathology* **130**, 123-133.

Fauquet, C., Fargette, D. & Thouvenel, J. C. (1988). Yield losses induced by African cassava mosaic virus in relation to the mode and date of infection. *Tropical Pest Management* **34**, 89-91.

Fauquet, C. & Fargette, D. (1990). African cassava mosaic virus: Etiology, epidemiology and control. *Plant Disease* **74**, 404-411.

Fauquet, C. M. & Stanley, J (2003). Geminivirus classification and nomenclanture: progress and problems. *Annals of Applied Biology* **142,** 165-189.

Fauquet, C .M., Bisaro, D. M., Briddon, R. W., Brown, J., Harrison, B. D., Rybicki, E. P.,
Stenger, D. C. & Stanley, J. (Study Group Chair). (2003). Revision of taxonomic criteria for species demarcation in the *Geminiviridae* family, and a new updated list of begomovirus species. *Archives of Virology* **148,** 405-421.

Fofana, I. B. F., Sangare, A., Ndunguru, J., Kahn, K. & Fauquet, C. M. (2003). Principals for control of virus diseases in developing countries In: Loebenstein, G and Thottappilly, G, *Virus and Virus-like diseases of major crops in developing countries.* Eds, Kluwer Academic Publishers, The Netherlands, UK.

Fondong, V., Pita, J. S., Rey, C., Beachy, R. N. & Fauquet, C. M. (1998). First report of the presence of East African cassava mosaic virus in Cameroon. *Plant Disease* **82**, 1172.

Fondong, V. N., Pita, S. T., Rey, M. E. C., de Kochko, A., Beachy, R. N. & Fauquet, C. M. (2000). Evidence of synergism between African cassava mosaic virus and a new double recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology* **81**, 287-297.

Fontes, E. O. B., Eagle, P. A., Sipe, P. A., Luckow, V. A. & Hanley-Bowdoin, L. (1994). Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. *Journal of Biological Chemistry* **269**, 8459-8465.

Frischmuth, T. & Stanley, J. (1998). Recombination between viral DNA and the transgenic coat protein gene of African cassava mosaic geminivirus. *Journal of General Virology* **79**, 1265-1271.

Gallitelli, D. & Accotto, G. P. (2001). Virus-resistant transgenic plants: potential impact on the fitness of plant viruses. *Journal of Plant Pathology* **83,** 3-9.

Gardner, W., Sunter, G., Brand, L., Elmer, J. S., Rogers, S. & Bisaro, D. (1988). Genetic analysis of tomato golden mosaic virus: the coat protein is not required for systemic spread or symptom development. *EMBO Journal* **7,** 899-904.

Gutierrez, G. (2000). DNA replication and cell cycle in plants: learning from geminiviruses. *The EMBO journal* **19**, 792-799.

Haley, A., Zhan, X. C., Richardson, K., Head, K. K. and Morris, B. (1992). Regulation of the activities of African cassava mosaic promoters by the AC1, AC2, and AC3 gene products. *Virology*, **188**, 905-909.

Hamilton, A. J. & Baulcombe, D. C. (1999). A novel species of small antisense RNA in posttranscriptional gene silencing. *Science* **286,** 950-952.

Hahn, S. K., Terry, E. R. & Leuschner, K. (1980). Breeding cassava for resistance to cassava mosaic disease. *Euphytica* **29**, 673-683.

Hanley-Bowdoin, L., Settlage, S. B., Orozco, B. M., Nagar, S. & Robertson, D. (1999). Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences* **18**, 71-106.

Hanley-Bowdoin, L., Settlage, S. B. & Robertson, D. **(2004).** Reprogramming plant gene expression: A prerequisite to geminivirus DNA replication. *Molecular Plant Pathology* **5,** 149-156.

Harris, K. F. (1982). Auchenorrhynchous vectors of plant viruses: Virus-vector interactions and transmission mechanisms. In: Proceedings of the international workshop on biotaxonomy, classification and biology of leafhoppers and plant hoppers (Auchenorrhyncha) of economic importance. 4-7 October 1982, London.

Harrison, B. D., Barker, H., Bock, K. R., Guthrie, E. J., Meredith, G. & Atkinson, M. (1977). Plant viruses with circular single-stranded DNA. *Nature* **270**, 760-762.

Harrison, B. D. (1985). Advances in geminivirus research. *Annual Review of Phytopathology* **23**, 55-82.

Harrison, B. D., Lennon, A. M., Massalski, P. R., Robinson, D. J. & Thomas, J. E. (1987). *Geographical variation in geminivirus isolates associated with cassava mosaic disease*. In: *Report of the Scottish Crop Research Institute for 1986*. pp. 176-180. Scottish Crop Research Institute. Invergowrie, UK.

Harrison, B. D. & Robinson, D. J. (1988). Molecular variation in vector-borne plant viruses: Epidemiological significance. *Philosophical Transactions of the Royal Society of London Series B* **321**, 447-462.

Harrison, B. D., Zhou, X., Otim-Nape, G. W., Liu, Y. & Robinson, D. J. (1997). Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Annals of Applied Biology* **131**, 437-448.

Henry, G., Westby, A. & Cillinson, C. (1998). *Global cassava end-uses and markets: Current situation and recommendations for further study.* Report of FAO consultancy by the European Group on Root, Tuber and Plantain: Internet htp//www.globalcassavastrategy.net/phase_i_market_study.html Access. 6/6/2000

Heyraud, F., Matzeit, V., Kamman, M., Schaefer, S., Schell, J. & Gronenborn, B. (1993a). Identification of the initiation sequence for viral-strand DNA synthesis of wheat dwarf virus. *EMBO Journal* **12**, 4445-4452.

Hillocks, R. J., Raya, M. D. & Thresh, J. M. (1996). The association between root necrosis and aboveground symptoms of brown streak virus infection of cassava in southern Tanzania. *International Journal of Pest Management* **42,** 285-289.

Hillocks, R. J., Raya, M., Mtunda, K. & Kiozia, H. (2001). Effects of brown streak virus disease on yield and quality of cassava in Tanzania. *Journal of Phytopathology* **149,** 1-6.

Hillocks, R. J (2003). Cassava brown streak virus disease: Summary of present knowledge on distribution, spread, effect on yield and methods of control. In: Legg J.P and Hillocks, R. J *Cassava Brown Streak Virus Disease: Past Present and Future. Proceedings of an International Workshop, Mombasa, Kenya,* 27-30 October 2002. Eds, Natural Resource International limited, Aylesford, UK.

Hong, Y. G., Robinson, D. J. & Harrison, B. D. (1993). Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. *Journal of General Virology* **74**, 2437-2443.

Hong, Y. & Stanley, J. (1995). Regulation of African cassava mosaic virus complimentary-sense gene expression by N-terminal sequences of the replication-associated protein AC1. *Journal of General Virology* **76**, 2415-2422.

Hou, Y-M. & Gilbertson, R. L. (1996). Increased pathogenicity in a pseudorecombinant bipartite geminivirus correlates with intermolecular recombination. *Journal of Virology* **70,** 5430-5436.

Jennings, D. L. (1970). *Cassava in East Africa.* pp 64-65. Proceedings of the 2nd International Tropical Root and Tuber Crops. August _1970. University of Hawaii.

Jennings, D. L. (1994). Breeding for resistance to African cassava mosaic geminivirus in East Africa. *Tropical Science* **34**, 110-122.

Jeffrey, J. L., Pooma, W. & Petty, I. T. D. (1996). Genetic requirements for local and systemic movement of tomato golden mosaic virus in infected plants. *Virology* **223**, 208-218.

Jones, W. O. (1959). *Manioc in Africa*. Stanford University Press, Stanford.

Jupin, I., de Kouchkovsky, F., Jouanneau, F. & Gronenborn, B. (1994). Movement of tomato yellow leaf curl geminivirus (TYLCV): Involvement of the protein encoded by ORF C4. *Virology* **204**, 82-90.

Kammann, M., Schalk, H.-J., Martzeit, V., Schaefer, S., Schell, J. & Gronenborn, B. (1991). DNA replication of wheat dwarf virus, a geminivirus, requires two cis-acting signals. *Virology* **184,** 786-790.

Kong, L., Orozco, B. M., Roe, J. L., Nagar, S., Ou, S., Feilar, H. S., Duffee, T., Miller, A. B., Gruissem, W., Robertson, D. & Hanley-Bowdoin, L. (2000). A geminivirus replication protein interacts with the retinoblastoma protein through a novel domain to determine symptoms and tissue specificity of plants. *The EMBO Journal* **19**, 3485-3495.

Kong, L. J. & Hanley-Bowdoin, L. (2002). A geminivirus replication protein interacts with a protein kinase and a motor protein that display different expression patterns during plant development and infection. *Plant Cell* **14,** 1817-18-1832.

Kufferath, H. & Ghesquière, J. (1932). La mosaique du manioc. *Compte-Rendu de la Société de Biologie Belge* **109,** 1146-1148.

Laufs, J., Schumacher, S., Geisler, N., Jupin, I. & Gronenborn, B. (1995a). Identification of the nicking tyrosine of geminivirus rep protein. *FEBBS Letters* **377**, 258-262.

Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S. G., Schell, J. & Gronenborn, B. (1995b). *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. *Proceedings of the American Academy of Sciences* **92**, 3879-3883.

Lazarowitz, S. G., Wu, L. C., Rogers, S. G. & Elmer, J. S. (1992). Sequence-specific interaction with viral AL1 protein identifies a geminivirus DNA replication origin. *Plant Cell* **4**, 799-809.

Legg, J. P. & Ogwal, S. (1998). Changes in the incidence of African cassava mosaic geminivirus and the abundance of its whitefly vector along south-north transects in Uganda. *Journal of Applied Entomology* **122,** 169-178.

Legg, J. P. & Raya, M. D. (1998). Survey of cassava virus disease in Tanzania. *International Journal of Pest Management* **44**, 17-23.

Legg, J. P. (1999). Emergence, spread and strategies for controlling the pandemic of cassava mosaic disease in eastern and central Africa. *Crop Protection* **18**, 627-637.

Legg, J. P. & Okao-Okuja, G. (1999). *Progress in the diagnosis and epidemiological characterisation of cassava mosaic geminiviruses in East Africa*. pp. 74-75. Proceedings of the VIIth International Plant Virus Epidemiology Symposium, April 11-16, 1999 (Abstract). Aguadulce (Almeria), Spain,

Legg, J. P., Okao-Okuja, G., Mayala, R. & Muhinyuza, J-B. (2001). Spread into Rwanda of the severe cassava mosaic virus disease pandemic and the associated Uganda variant of East African cassava mosaic virus (EACMV-Ug). *Plant Pathology* **50,** 796.

Legg, J. P. & Thresh, M. J. (2003). Cassava virus disease in Africa. pp. 517-555. In: *Proceedings of the First International Workshop on Plant Virology in Sub-saharan Africa*. IITA, Ibadan, Nigeria.

Legg, J. P. & Fauquet, C.M (2004). Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* (In press).

Maule, A. J. (1991). Virus movement in infected plants. *Critical Review of Plant Science* **9**, 457-473.

Maruthi, M. N., Colvin, J., Seal, S. & Thresh, J. M. (2002). First report of a district begomovirus infecting cassava in Zanzibar. *Plant Disease* **86**, 187.

MDB (Marketing Development Bureau). (1985). *Price policy recommendation for the 1895* Agricultural Price Review. Ministry of Agriculture and Livestock Development, Dar-Es- Salaam, Tanzania.

Monci, F., Navas-Castillo, J. & Moriones, E. (2001). Evidence of natural occurring recombinant between tomato yellow leaf curl virus and tomato yellow leaf curl Sardiana virus in Spain. *Plant Disease* **85**,1289.

Monger, W. A., Seal, S., Isaac, A. M. & Harrison, B. D. (2001). Molecular characterization of cassava brown streak virus coat protein. *Plant Pathology* **50,** 527-534.

Morales, F. J. & Anderson, P. K. (2001). The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. *Archives of Virology* **146**, 415-441.

Morris, B., Richardson, K., Eddy, P., Zhan, X. C., Haley, A. & Garder, R. (1991). Mutagenesis of the AC3 open reading frame of African cassava mosaic virus DNA reduces DNA-B replication and ameliorates disease symptoms. *Journal of General Virology* **72**, 1205-1213.

Msabaha, M. A. M., Ndibaza, R. E. & Nyango, A. K. (1988). *Cassava research in*

Tanzania for the period 1930-1988. Pg. 25 Tanzania Agricultural Research Organisation. Ministry of Agriculture and Livestock Development, Dar Es Salaam, Tanzania.

Ndunguru, J., Sseruwagi, P., Jeremiah, S. & Kapinga, R. (2003). Whiteflies as vectors of plant viruses in cassava and sweetpotato in Africa: Tanzania. In: Anderson, P. K and R. H. Markham, *Whiteflies and whitefly-borne viruses in the tropics: building a knowledge base for global action*. Eds, Centro Internacional de Agricultura Tropical, Cali, Colombia.

Neuenschwander, P., Hughes, J. d'A., Ogbe, F., Ngatse, J. M. & Legg, J. P. (2002). The occurrence of the Uganda Variant of East African Cassava Mosaic Virus (EACMV-Ug) in western Democratic Republic of Congo and the Congo Republic defines the westernmost extent of the CMD pandemic in East/Central Africa. *Plant Pathology* **51,** 384.

Noueiry, A. O., Lucas, W. J. & Gilbertson, R. W. (1994). Two proteins of a plant DNA virus co-ordinate nuclear and plasmodesmal transport. *Cell* **76**, 925-932.

Novick, R. P. (1998). Contrasting lifestayles of rolling-circle phages and plasmids. *Trends in Biochemistry Science* **23,** 434-438.

Ogbe, F. O., Songa, W. & Kamau, J. W. (1996). Survey of the incidence of African cassava mosaic and East African cassava mosaic viruses in Kenya and Uganda using a monoclonal antibody based diagnosis test. *Roots* **3**, 10-13.

Ogbe, F. O., Legg, J., Raya, M. D., Muimba-Kankolongo, A., Theu, M. P., Kaitisha, G., Phiri, N. A. & Chalwe, A. (1997). Diagnostic survey of cassava mosaic viruses in Tanzania, Malawi and Zambia. *Roots* **4**, 12-15.

Orozco, B. M. & Hanley-Bowdoin, L. (1996). A DNA structure is required for geminivirus replication origin function. *Journal of Virology* **70,** 148-158.

Ogbe, F. O.,Thottappilly, G., Dixon, A. G. O. & Mignouna, H. D. (2003). Variants of *East `African cassava mosaic virus* and its distribution in double infections with *African cassava mosaic virus* in Nigeria. *Plant Disease* **87,** 229-232.

Otim-Nape, G. W., Bua, A., Thresh, J. M., Baguma, Y., Ogwal, S., Semakula, G. N., Acola, G., Byabakama, B. & Martin, A. (1997). *Cassava mosaic virus disease in Uganda: The current pandemic and approaches to control*. Natural Resources Institute, Catham, UK.

Otim-Nape, G. W., Alicai, T. & Thresh, J. M. (2001). Changes in the incidence and severity of cassava mosaic virus disease, varietal diversity and cassava production in Uganda. *Annals of Applied Biology* **138**, 313-327.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity**.** *Journal of General Virology* **76,** 25-35.

Padidam, M., Beachy, R. N. & Fauquet, M. (1999). A phage single-stranded DNA (ssDNA) binding protein complements ssDNA-Accumulation of geminivirus and interfers with viral movement. *Journal of Virology* **73**, 1609-1616.

Pascal, E., Goodlove, P. E., Wu, L. C. & Lazarowitz, S. G. (1993). Transgenic tobacco plants expressing the geminivirus BL1 protein exhibit symptoms of viral disease. *Plant Cell* **5**, 795-807.

Pascal, E., Sanderfoot, A. A., Ward, B. M., Medville, R., Targeon, R. & Lazarowtz, S. G. (1994). The geminivirus BR1 movement protein binds single-stranded DNA-And localises to the cell nuclear. *Plant Cell* **6**, 995-1006.

Pita, S., Fondong, V. N., Sangare, Otim-Nape, G. W., Ogwal, S. & Fauquet, C. M. (2001a). Recombination, pseudorecombination and synergism of geminiviruses are

determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology* **82**, 655-665.

Pita, J. P., Fondong, V. N., Sangare, A., Kokora, R. N. N. & Fauquet, C. M. (2001b). Genomic and biological diversity of the African cassava geminiviruses. *Euphytica* **120**, 115-125.

Plucknett, D. L., Phillips, P. T. & Kagbo, R. B. (Eds). (2000). *A global development strategy for cassava: Transforming a traditional tropical root crop. Surring rural industrial development and raising incomes for rural poor.* FAO Plant Production and Crop Protection Division, Rome, Italy.

Posada, D. & Crandall, K. A. (2001). Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. *Proceedings of the Natural Academic Sciences of USA* **98**, 13758-13762

Revington, G. N., Sunter, G. & Bisaro, B. M. (1989). DNA sequences essential for replication of the B genome component of tomato golden mosaic virus. *Plant Cell* **1**, 985-992.

Ridgemen, J. E., Krake, L.R., Rezaia, M. A. & Dry, I. B. (1994). ORF C4 of tomato leaf curl geminivirus is determinant of symptom severity. *Virology* **204**, 847-850.

Roberts, I. M., Robinson, D. J. & Harrison, B. D. (1984). Serological relationships and genome homologies among geminivirus. *Annals of Applied Biology* **105**, 483-493.

Rogers, S. G., Bisaro, D. M., Horsch, R. B., Fraley, R. T., Hoffmann, N. L., Brand, L., Elmer, J. C. & Lloyd, A. M. (1986). Tomato golden mosaic virus A component DNA replicates autonomously in transgenic plants. *Cell* **45**, 593-600.

Rojas, M. R., Gilbertson, R. L., Russell, D. R. & Maxwell, D. P. (1993). Use of

degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminivirus. *Plant Disease* **77**, 340-347.

Sanderfoot, A. A. & Lazarowtz, S. G. (1995). Cooperation in viral movement: The geminivirus virus BL1 movement protein interacts with BR1 and directs it from the nuclear to the cell periphery. *The Plant Cell* **7**, 1185-1194.

Sanderfoot, A. A., Ingham, D. J. & Lazarowitz, S. G. (1996). A viral movement protein gene as a nuclear shuttle. *Plant Physiology* **110,** 23-33.

Saunders, L., Lucy, A. & Stanley, J. (1991). DNA forms of geminivirus African cassava mosaic virus consistent with a rolling circle mechanism replication. *Nucleic Acid Research* **19**, 2325-2330.

Saunders, K., Lucy, A. & Stanley, J. (1992). RNA-primed complementary-sense DNA synthesis of the geminivirus African cassava mosaic virus**.** *Nucleic Acid Research* **20,** 6311-6315.

Saunders, K., Salim, N., Mali, V.R., Malathi, V.G., Briddon, R., Markham, P. & Stanley, J., (2002). Characterisation of Sri Lankan Cassava Mosaic Virus and Indian Cassava Mosaic Virus: Evidence of Acquisition of a DNA B Component by a Monopartite Begomovirus. *Virology* **295,** 63-74.

Seif, A. A. (1982). Effect of cassava mosaic virus on yield of cassava. *Plant Disease* **66**, 661-662.

Settlage, S. B., Miller, A. B. & Hanley-Bowdoin, L. (1996). Interactions between geminivirus replication proteins. *Journal of Virology* **70,** 6790-6795.

Stanley, J. (1983). Infectivity of the cloned geminivirus genome requires sequences from both DNAs. *Nature* **301**, 643-645.

Stanley, J. & Gay, M. R. (1983). Nucleotide sequence of cassava latent virus DNA. *Nature* **301**, 260-362.

Stanley, J., Markham, P. G., Callis, R. J. & Pinner, M. S. (1986). The nucleotide sequence of an infectious clone of the geminivirus beet curly top virus. *EMBO Journal* **5,** 1761-1767.

Stanley, J. & Townsend, R. (1986). Infectious mutants of cassava latent virus generated in vivo from intact recombinant DNA clones containing single-copies of the genome. *Nulceic Acid Research* **14,** 5981-5998.

Stanley, J. & Latham, J. R. (1992). A symptom variant of beet curl top geminivirus produced by mutation of open reading frame C4. *Virology* **190**, 506-509.

Stanley, J. (1995). Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs with the conserved motif during the initiation of rolling circle DNA replication. *Virology* **206**, 707-712.

Stenger, D. C., Revington, G. N., Stevenson, M. C. & Bisaro, D. M. (1991). Replicational release of geminivirus genomes from tandemly repeated copies: Evidence for rolling-circle replication of a plant viral DNA. *Proceedings of Natural Academic Science of USA* **88**, 8029-8033.

Storey, H. H. (1936). Virus diseases of East African Plants. VI. A progress report on studies of the diseases of cassava. *East African Agricultural Journal* **2**, 34-39.

Storey, H. H. & Nichols, R. F. W. (1938). Studies of the mosaic disease of cassava. *Annals of Applied Biology* **25**, 790-806.

Sunter, G., Gardiners, W. E., Rushing, A. E., Rogers, S. G. & Bisaro, D. M. (1987).

Independent encapsidation of tomato golden mosaic virus A component DNA in transgenic plants. *Plant Molecular Biology* **8**, 477-484.

Sunter, G., Hartitz, M. D., Hormuzdi, S. G., Brough, C. L. & Bisaro, M. D. (1990). Genetic analysis of tomato golden mosaic virus: ORF AL2 is required for coat protein accumulation while ORF AL3 is necessary for efficient DNA replication. *Virology* **179**, 69-77.

Sunter, G. & Bisaro, D. M. (1992). Transactivation of geminivirus AR1 and BR1 gene expression by the AL2 gene product occurs at the level of transcription. *Plant Cell* **4**, 1321-1331.

Swanson, M. M. & Harrison, B. D. (1994). Properties, relationships and distribution of cassava mosaic geminiviruses. *Tropical Science* **34**, 15-25.

Tidbury, G. E. (1937). A note on the yield of mosaic-diseased cassava. *The East African Agricultural Journal* **3**, 119.

Thresh, J. M. (1988). Strategies for controlling African cassava mosaic virus. In: *Proceedings of the International Seminars on African cassava mosaic disease and its control*, pp. 26-35, 4-8th May 1987. CTA/FAO/ORSTOM/IITA/IAPAC, Yamoussoukro, Cốte d'Ivoire.

Thresh, J. M., Fargette, D. & Otim-Nape, G. W. (1994). Effects of African cassava mosaic virus on the yield of cassava. *Tropical Science* **26**, 34-37.

Thresh, J. M., Otim-Nape, G. W., Legg, J. P. & Fargette, D. (1997). African cassava mosaic disease: The magnitude of the problem. *African Journal of Root and Tuber Crops* **2**, 13-19.

Thresh, J. M., Otim-Nape, G. W., Legg, J. P. & Fargette, D. (1998). *The control of*

African cassava mosaic disease: Phytosanitaion and /or resistance. Pp. 670-677. Edited by A. haddidi, R. K. Khetarpal. and H. Koganezawa. Plant Virus Disease Control. A. P. S. Press, St. Paulo.

Thresh, J. M. & Mbwana, M. W. (1998). Cassava mosaic and cassava brown streak disease in Zanzibar. *Roots Newsletter* **5**, 6-9.

Townsend, R., Stanley, J., Curson, S. J. & Short, M. N. (1985). Major polyadenylated transcripts of cassava latent virus and location of the gene encoding coat protein. *The EMBO Journal* **4**, 33-37.

Townsend, R., Watts, J. & Stanley, J. (1986). Synthesis of viral DNA forms in *Nicotiana plumbaginifolia* protoplasts inoculated with cassava latent virus (CLV): Evidence for independent replication of one component of the CLV genome. *Nucleic Acid Research* **14,** 1253-1255.

Thomas, J. E., Massalski, P. R. & Harrison, B. D. (1986). Production of monoclonal antibodies to African cassava mosaic virus and the differences in their reactivity with other whitefly-transmitted geminiviruses. *Journal of General Virology* **67,** 2739-2748.

Unseld, S., Frischmuth, T. & Jeske, H. (2004). Short deletions in nuclear targeting sequences of African cassava mosaic virus coat protein prevent geminivirus twinned particle formation. *Virology* **318,** 90-101.

Vanitharani, R., Chellappan, P. & Fauquet, C. M. (2003). Short interfering RNA-mediated interference of gene expression and viral DNA-Accumulation in cultured plant cells. *Proceedings of Natural Academic Science of USA* **100**, 9632-9636.

von Arnim, A., Frischmuth, T. & Stanley, J. (1993). Detection and possible functions of African cassava mosaic virus DNA-B gene products. *Virology*, **192**, 264-272.

Ward, B. M., Medville, R., Lazarowitz, S. G. & Turgeon, R. (1997). The geminivirus BL1 movement protein is associated with endoplasmic reticulum derived tubules in developing phloem cells. *Journal of Virology* **71**, 3726-3733.

Warburg, O. (1894). Die Kulturpflanzen Usambaras. Mitt. Dtsch Schutzgebietens, **7,** 131.

Zhan, X., Haley, A., Richardson, K. & Moris, B. (1991). Analysis of the potential promoter sequences of African cassava mosaic virus by transient expression of the β-glucuronidase gene. *Journal of General Virology* **72**, 2849-2852.

Zhang, P. & Gruissem, W. (2003). Efficient replication of cloned African cassava mosaic virus in cassava leaf disks. *Virus Resarch* **92,** 47-54.

Zhou, X., Liu, Y., Calvert, L., Munoz, C., Otim-Nape, G. W., Robinson, D. J. & Harrison, B.D (1997). Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has risen by interspecific recombination. *Journal of General Virology* **78,** 2101-2111.

Zhou, X., Robinson, D. J. & Harrison, B. D. (1998). Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania. *Journal of General Virology* **79,** 2835-2840.