African Journal of Biotechnology Vol. 7 (14), pp. 2353-2358, 18 July, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

The use of biolistic inoculation of cassava mosaic begomoviruses in screening cassava cultivars from Ghana for resistance/susceptibility to cassava mosaic disease

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Accepted 11 June, 2008

The resistance/susceptibility of 5 cassava cultivars from Ghana to infectious clones of two cassava mosaic viruses (ACMV-[CM] and EACMV/ACMV-[CM]) was investigated in this study. Plantlets of cassava cultivars were obtained using nodal cuttings initiated from tissue culture. These cassava cultivars were challenged with both DNA A and B components of the infectious clones named above using particle gun bombardment. The cassava cultivars showed varying degrees of susceptibility/resistance to the two infectious clones used. All symptoms of Cassava Mosaic Disease (CMD) observed were systemic in nature. Generally, all cassava cultivars used in this study exhibited varying degrees of recovery from virus infection. This report demonstrates the ability of using biolistic technology to screen cassava cultivars for tolerance/resistance and that it may be used to recommend resistant cultivars to the farming community.

Key words: Biolistic, cassava, ACMV, EACMV, inoculation.

INTRODUCTION

Cassava mosaic disease (CMD) is the most important factor limiting cassava yields in many parts of Africa (Fargette et al., 1988; Fauquet and Fargette 1990; Legg and Fauquet 2004) and it is responsible for an estimated loss of yield of over 1.5 billion US dollars a year (Thresh et al., 1994). In Africa, cassava mosaic disease is caused by two whitefly-transmitted geminiviruses, *Africa cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) (Hong et al., 1993; Swanson and Harrison, 1994; Deng et al., 1997; Zhou et al., 1998 and Pita et al., 2001). The ACMV and EACMV are members of the *Geminiviridae*, genus *Begomovirus* (Fauquet and Stanley, 2003; Fauquet et al., 2005).

Most *begomoviruses* have bipartite circular DNA genomes, referred to as DNA-A and DNA-B components. A few species have only a single genomic component resembling DNA-A (Zhou et al., 2003). DNA-A encodes all viral proteins necessary for replication and encapsidation of both components (Rogers et al., 1986) while DNA-B component encodes for proteins necessary for efficient systemic spread of the virus throughout the plant (Ingham et al., 1995). It is possible for ACMV and EACMV to co-infect plants. Presumably, due to the combined action of the posttranscriptional gene silencing (PTGS) suppressors AC4 and AC2, from ACMV and EACMV, respectively, a co-infected cassava plant produce increased mosaic symptoms, a phenomenon called synergistic interaction (Vanitharani et al., 2004; Vanitharani et al., 2005). Fondong et al. (2000) reported a synergistic interaction between two ACMV and EACMV strains from

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Abbreviations: CMD, Cassava mosaic disease; ACMV, African cassava mosaic virus; EACMV, East African cassava mosaic virus; ACMV-CM, African cassava mosaic virus-Cameroon; EACMV-CM, East African cassava mosaic virus-Cameroon; dpi, days post inoculation.

Cameroon (ACMV-[CM] and EACMV-[CM]), where cassava plants co-infected with these two viruses developed more severe symptoms as compared to plants with single infection.

CMD is widespread throughout all cassava growing regions in Ghana and neighbouring countries such as Benin, Nigeria, Cameroon, Senegal and Guinea (Nweke et al., 2002; Wydra and Msikita 1998; Okao-Okuja et al., 2004). The emergence of EACMV, which has its origin from East Africa but has been documented in Central and West Africa (Fondong et al., 1998; Offei et al., 1999; Ogbe et al., 1999), raises a lot of concern to cassava growers in the sub region. The uncontrolled spread of CMD may be partially responsible for the low yield of 13.1 tonnes/ hectare averagely produced by a Ghanaian farmer. In Ghana, an elaborate programme is in place where elite cassava varieties, displaying mild CMD symptoms are being multiplied and distributed to local farmers. It is not clear whether these varieties really are tolerant to CMD or if the mild symptoms are due to the absence of white-flies in the cultivated area. As with all resistant plants, there is a danger of resistance breakdown, especially under high pathogen pressure. If such a breakdown occurs, it may wipe out entire cassava fields as has already occurred in East Africa with the emergence of a virulent strain of EACMV found in Uganda (Harrison et al., 1997; Legg, 1999). To address the issue of whether the elite varieties are tolerant or not, we have challenged five cassava cultivars from Ghana with an infectious clone of ACMV-[CM], or a mixture of ACMV-[CM] and EACMV-[CM] infectious clones, using particle gun bombardment. This method may be used to evaluate other cultivars that are being considered for field production by virtue of their tolerance/resistance to begomoviruses.

MATERIAL AND METHODS

Source of cassava plants

For ACMV-CM inoculation, a total of six (6) cassava cultivars were used; Biafra (BF), Abasa fitaa (ABA), Ankrah (ANK), Bosome nsia (BN) and Gblemaduade (GBL) from the germplasm collection at The Biotechnology and Nuclear Agriculture Research Institute (BNARI-Ghana) and TMS 60444 was kindly donated by International Laboratory for Tropical Agriculture Biotechnology (ILTAB).

For ACMV-[CM]/EACMV-[CM] mixed inoculation trials, cultivars TMS 60444, ABA, GBL and BF were used.

Regeneration from nodal cuttings

Nodal cuttings from cultivars mentioned above were sterilized using bleach followed by 3 subsequent washings in sterile distilled water. The nodal cuttings were grown in modified Murashige and Skoog (MS) medium supplemented with 2 µM of 6-Benzylaminopurine (BAP). After 3 - 4 weeks in culture, the shoots obtained were transferred to soil for rooting and hardening. Shoots obtained were used for biolistic bombardment trials after reaching a height 25 cm.

Inoculation with viral DNA

The infectious clones of ACMV-[CM] and EACMV-[CM] used in this study were kindly supplied by (ILTAB). 30 mg of gold particles (Biorad, Hercules, California), were added to 500 µl of 100% ethanol and centrifuged at 1200 rpm. Supernatant was removed and 500 µl of 100% ethanol was added and centrifuged again. Supernatant was discarded and particles were resuspended in 500 µl of distilled water by vortexing and then pelleted. The DNA A and B components of ACMV-[CM] and for double infection DNA A and B components ACMV-[CM] and EACM-[CM] were added to the gold particles during slow vortexing to obtain 100 ng DNA per shot per plant. Whilst vortexing, 20 µl spermidine (0.05 M) and 50 µl $CaCl_2$ (1 M) were added in succession. The mixture was left at room temperature for 10 min and pelleted. Particles were resuspended in 50 µl of cold 100% ethanol. 10 µl of the suspension were distributed onto the macrocarriers. The Particle Delivery System PDS1000/He biolistic device (Biorad) was used for bombardment at a pressure of 1500 psi. Five plants from each cultivar GLB, BF, BN, ABA, ANK, and TMS 60444 were bombarded with DNA A and B from ACMV-[CM]. For the ACMV-[CM] and EACMV-[CM], five plants each of TMS 60444, ABA, GBL and BF were bombarded. All cassava-inoculated seedlings with the various infectious clones were grown in a greenhouse at 28°C and 16 h photoperiod. The symptoms on leaves were scored in accordance with the criteria described by Fauquet and Fragette (1990) where a 0 to 5 point scale was used with 0 meaning no symptom and 5 denoting the most severe symptom.

DNA extraction and southern blotting analysis

Total DNA was extracted from plants according to the method described by Dellaporta et al. (1983). Phenol-chloroform extraction was carried out to remove excess protein before Southern blot analysis. DNA concentrations were measured by a spectrophotometer. 8 µg of total DNA were loaded and separated on two 1.2% agarose gel stained with ethidium bromide and subsequently transferred to Hybond-N+ membranes (Amersham). The probe used in the southern blotting analysis was an excised and purified *EcoRI-BamHI* (nt1714-140) fragments from ACMV DNA-A. The probe was labeled with [^{32}P] dATP by random priming, as described by Sambrook et al. (1989). Signals were detected by X-ray film exposure. Both membranes were exposed for the same amount of time. For ACMV-CM infection, the probe was used to analyse viral load 14 and 28 days post inoculation (dpi). For ACMV-CM/EACMV-CM double infection the viral load was accessed 28 and 40 dpi using the same probe.

RESULTS

ACMV symptoms

Regardless of cassava cultivar type, 80 - 100% of the inoculated plants developed symptoms after single infection. All symptoms were systemic and leaves showed “mosaic” patches in all cultivars, especially in BF (Arrow-Figure 1). Initial symptoms after ACMV single infection was observed 12 - 15 dpi, with an average severity score of 2.5. All cultivars except ABA showed symptoms (Figure 1A - F) at 14 dpi ACMV-[CM]. ABA showed initial symptoms at 17 dpi. At 28 dpi, TMS 60444, ANK and GBL (Figure 1G, J and L) showed signs of recovery from ACMV single infection compared to BF (Figure 1I) where

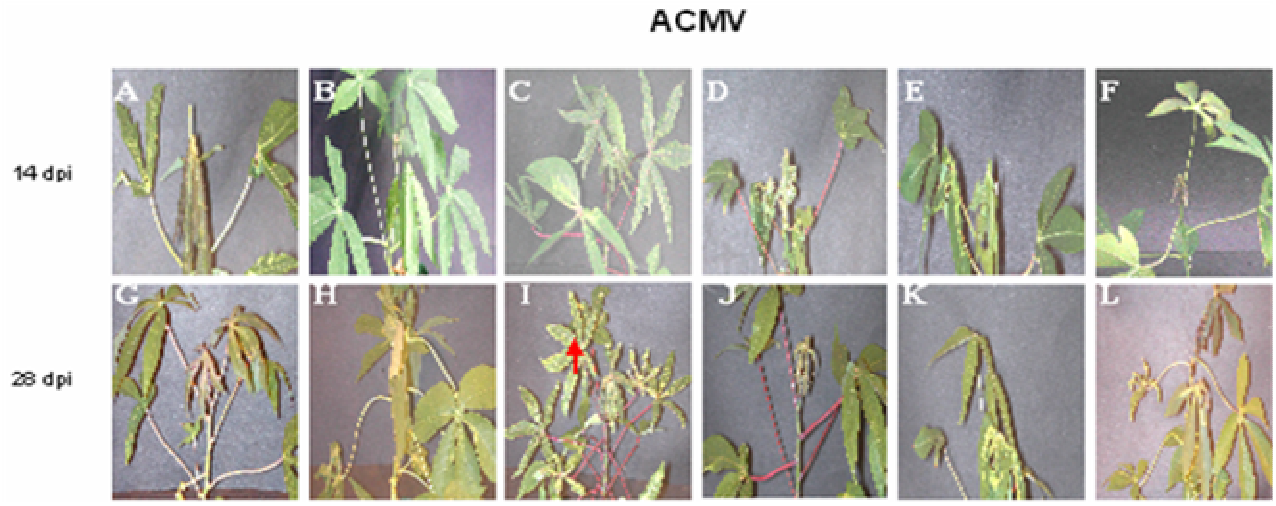

Figure 1. Symptoms of CMD on six cassava cultivars bombarded with the ACMV-CM infectious clone. 14 dpi: (A) Tms 60444, (B) ABA, (C) BF, (D) ANK, (E) BN and (F) GBL. 28 dpi: (G) TMS 60444, (H) ABA, (I) BF, (J) ANK, (K) BN and (L) GBL. Mosaic symptoms are shown with an arrow.

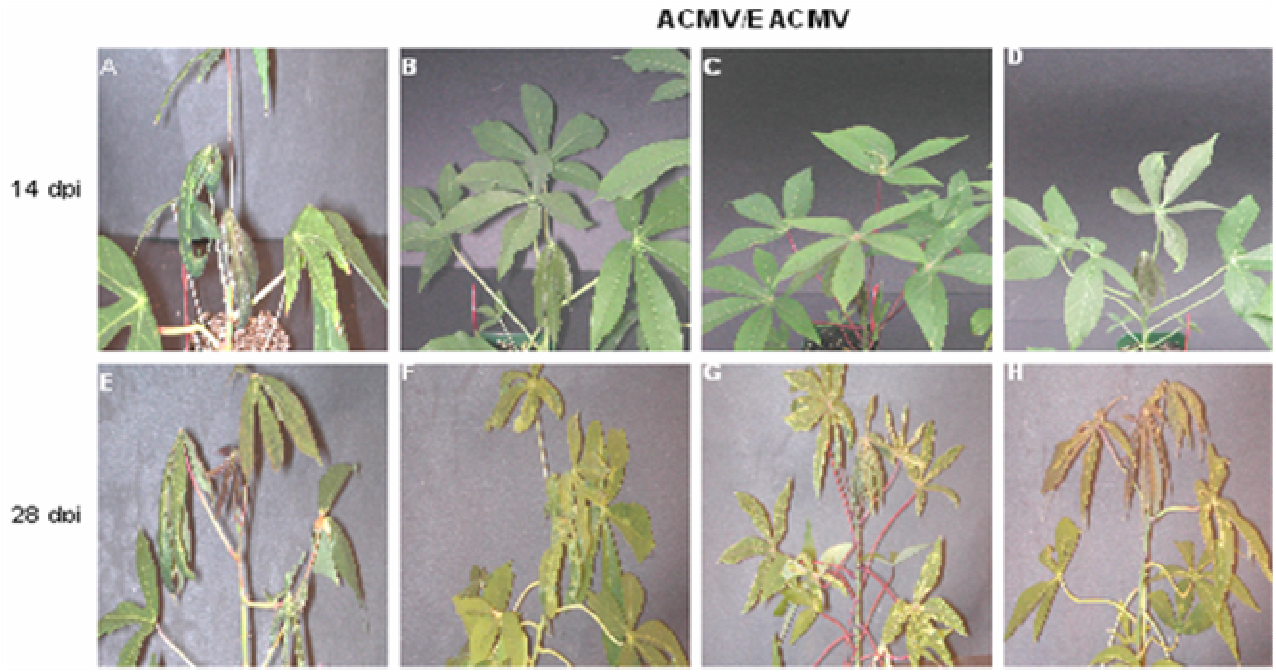

Figure 2. Symptoms of CMD on four cassava plants bombarded with mixed double infection of ACMV-CM and EACMV-CM.14 dpi: (A) TMS 60444, (B) ABA, (C) BF, (D), GBL. 28 dpi: (E) TMS 60444, (F) ABA, (G) BF and (H) GBL.

mosaic symptoms persisted. TMS 60444 and BF displayed most severe symptoms with a severity score 4.0. However, TMS 60444 attained severity score 4.0 after 24 dpi while BF attained the same score after 50 dpi. Cultivar ABA showed recovery from 2.2 at 30 dpi compared to 1.4 at 50 dpi. BN reached a maximum score of 3.0 at 38 dpi compared to 1.0 at 56 dpi. BN and TMS 60444 exhibited the best ability to recover from ACMV.

ACMV/EACMV symptoms on cassava

Symptom development upon mixed infection was first detected at 20 dpi in ABA, BF and GBL (Figure 2 B - D). This was delayed compared to single infection where it was detected at 14 dpi (BF and GBL) and 17 dpi (ABA). Double infected TMS 60444 showed initial mosaic symptoms at 14 dpi. TMS 60444, GBL and BF reached

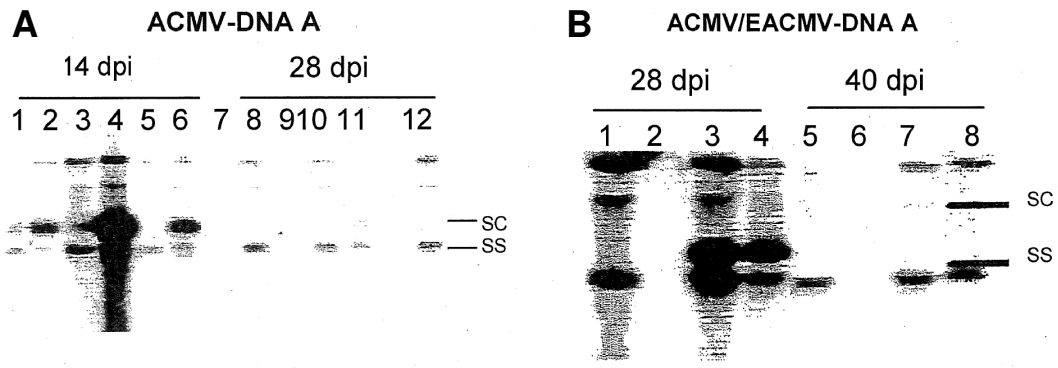

Figure 3. A. Southern blot analysis to show viral loads of ACMV-DNA A (left). Lanes 1 - 6 show viral load after 14 dpi in TMS 60444, ABF, BF, ANK, BN and GBL respectively. Lane 7 is empty. Lanes 8 - 12 shows viral load after 28 dpi in Tms 60444, ABA, BF, ANK, BN and GBL respectively. The positions of single-stranded (ss) and supercoiled (sc) DNAs are indicated. B. Southern blot analysis to show viral loads of ACMV-DNA A component in double infection. Lanes 1 - 4 represent viral load after 14 dpi in TMS 60444, ABA, BF, GBL respectively. Lanes 5 - 8 shows viral load after 28 dpi in TMS 60444, ABA, BF, GBL respectively. The positions of single-stranded (ss) and supercoiled (sc) DNAs are indicated.

their highest severity score of 3.5 after 21, 38 and 36 dpi respectively. All the four cultivars had recovered from the double infection of EACMV/ACMV after 41 dpi.

Southern blotting analysis

Blot analysis showed that at 14 dpi, ANK (Figure 3A-lane 3) and BF (Figure 3A-lane 4) gave the highest accumulation of viral DNA in single infection. After 28 days, viral DNA accumulation was low in TMS 60444, ABA, ANK and GBL (Figure 3A, lanes 8-12). The results of southern blotting analysis conducted with total DNA from EACMV/ACMV mixed infection seem to show that there were higher levels of ACMV-A accumulation after 28 dpi for cultivars TMS 60444, BF and GBL (Figure 3B, lanes 1, 3 - 4 respectively) as compared to lower levels of viral accumulation after 40 dpi (Figure 3B, lanes 5 - 8). No ACMV-A accumulation was detected in cultivar ABA (Figure 3B, lanes 2 and 6).

DISCUSSION

The use of biolistics delivery of viral DNA may speed up the evaluation of the tolerance/resistance of cassava cultivars in breeding programmes as traditional infection by the whiteflies is time consuming and laborious. Biolistic mediated infection may assist in determining the level of resistance/tolerance to CMD cassava breeding lines. This is valuable as reduced CMV sensitivity is an important goal in cassava breeding.

The results obtained here showed the synergistic nature of the double infection with ACMV-[CM] and EACMV-[CM] with respect to the cultivars used in this work. The degree and differences in symptom severity of cassava breeding lines infected by the infectious clones varied, suggesting that cassava breeding cultivars used in this study varied in their tolerance to the infectious clones used. Variation in cassava cultivars genetic ability to resist CMD has also been reported by other studies (Lapidot and Friedmann, 2002; Ogbe et al., 2002). For ACMV-CM inoculation, TMS 60444 and BF were highly susceptible to the virus whereas ABA and GBL were less susceptible. There was a significant difference between cultivars that were infected with ACMV-CM alone and double infection of ACMV-CM/EACMV-CM. With the exception of GBL, symptom severity score for ABA and BF infected with the double infectious clone of ACMV-CM/EACMV-[CM] were higher than their corresponding values for single ACMV-[CM] infection. This shows evidence of synergism between the two viruses that result in an increase in symptom severity (Fondong et al., 2000; Hamson et al., 1997). Most of the cultivars used reached their maximum severity score after 4-5 weeks post inoculation.

Generally, virus accumulation was higher at 14 dpi and 28 dpi in single and double infected cassava cultivars respectively as evident by the thickened dark bands (Figure 3A and B).

In ACMV-[CM] inoculation, the cassava plants ability to recover was seen in the lower DNA-A levels at 28 dpi (Figure 3A-lanes 8 - 12) as compared to 14 dpi (Figure 3A-lanes 1 - 6). This phenomenon was also seen in double infection in cassava cultivars which were examined 40 dpi (Figure 3B-lanes 5 - 8) and 28 dpi (Figure 3B-lanes 1, 3 - 4), indicating an inherent genetic mechanism in cassava plants to recover after infection with cassava mosaic virus.

Southern blot analysis, though from two different nitrocellulose membranes, seem to reveal that viral loads in double infection (Figure 3B) enhanced increment in virus

concentration as against single infection (Figure 3B) as reported by Ariyo et al. (2006). It was also observed that ABA and GBL were moderately tolerant to both single infection (Figure 1B and H; Figure 1 F and L) and double infection (Figure 2 B and F and D and H) respectively. The explanation to this may be that the cassava cultivars ABA and GBL may be offspring from crosses involving cassava cultivars with high tolerance to the cassava mosaic virus. To this date, the search for a cassava cultivar with known resistance to begomoviruses has been embroiled in much controversy, though Akano et al. (2002) reported that a known local cassava landrace, TME 4 with a dominant '*R'* gene (CMD-2) showed resistance to CMD. Similar pattern of resistance/susceptibility has also been observed in some of the cultivars on cassava fields in Ghana (personal observation).

ABA, GBL and BF, which were recent introductions into the cassava germplasm in Ghana, have been considered more tolerant than the landrace cultivars. However, it does appear that this tolerance to CMD infection is being gradually broken down with time as recent observations on cassava fields have revealed (personal observation). Nevertheless, this study revealed that ABA may be considered best in terms of CMD tolerance of the cultivars analyzed here.

In conclusion, this work attempts to screen for tolerance/resistance against geminiviruses using a biolistic approach. Though laboratory conditions may differ from field condition, this technique is very fast and may speed up the evaluation of cassava cultivars to complement breeding programmes.

ACKNOWLEDGEMENTS

We thank USAID for the fellowship grant at the Donald Danforth Plant Science Centre, St. Louis-USA. We thank Dr. Claude Fauquet, Director of the International Laboratory for Tropical Agriculture Biotechnology (ILTAB) whose laboratory this work was carried out. Special thanks go to Dr. Nigel Taylor, Dr Vanitha Ramachandra and Dr. Chellappan Padmanabhan for their assistance. We further thank Professor J.H. Amoasi, Professor G.Y.P. Klu and Professor (Mrs) Victoria Appiah, all of the Ghana Atomic Energy Commission for their support.

REFERENCES

Akano AO, Dixon AGO, Mba C, Barrera E, Fregene M (2002). Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. Theor. Appl. Genet. 105: 521-525.

Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA mini preparation: version II. Plant Molecular Biology Reporter 1:19-21.

Deng D, Otim-Nape WG, Sangare A, Ogwal S, Beachy RN, Fauquet CM (1997). Presence of a new virus closely related to East African cassava mosaic geminivirus associated with cassava mosaic outbreak in Uganda. Afr. J. Root Tuber Crops 2: 23-28.

Fargette D, Fauquet CM, Thouvenel JC (1988). Yield losses induced by African cassava mosaic virus in relation to the mode and date of infection. Trop. Pest Manag. 34: 123-133.

Fauquet CM, Fargette D (1990). African cassava mosaic virus: etiology, Epidemiology and control. Plant Dis. 74: 404-411.

Fauquet C, Mayo M, Maniloff J, Desselberger U, Ball L, eds. (2005). *Virus Taxonomy VIIIth* Report of the International Committee on Taxonomy of Viruses. London: Elsevier/Academic.

Fauquet C, Stanley J (2003). Geminivirus classification and nomenclature progress and problems. Ann. Appl. Biol. 142: 165-189.

Fondong VN, Pita JS, Rey C, Beachy RN, Fauquet CM (1998). First report of the presence of East African cassava mosaic virus in Cameroon. Plant Dis. 82, 287-297.

Fondong VN, Pita JS, Rey MEC, DeKochko, Beachy RN, Fauquet CM (2000). Evidence of synergism between African cassava mosaic virus and a new double recombinant Geminivirus infecting cassava in Cameroon. J. Gen. Virol. 81: 287-297.

Harrison BD, Zhou X, Otim-Nape GW, Liu Y, Robinson DJ (1997). Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. Ann. Appl. Biol. 131: 437-448.

Hong YG, Robinson DJ, Harrison BD (1993). Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. J. Gen. Virol. 74: 2437-2443.

Ingham DJ, Pascal E, Lazarowitz SG (1995). Both bipartite geminivirus movement proteins define viral host range but only BL1 determines viral pathogenicity. Virology 265: 218-225.

Lapidot M, Friedmann M (2002). Breeding for resistance to whitefly transmitted geminiviruses. Ann. Appl. Biol. 140: 109-127.

Legg JP (1999). Emergence spread and strategies for controlling the pandemic of cassava mosaic virus disease in east and central Africa. Crop Prot. 18: 627-637.

Legg JP, Fauquet CM (2004). Cassava mosaic geminiviruses in Afr. Plant Mol. Biol. 56: 585-599

Nweke FI, Spencer SCD, Lynam KJ (2002). The cassava transformation: Africa's best kept secret. Michigan State University Press. East Lansing,Michigan. 48823-5202. p. 273.

Offei SK, Owuna-Kwakye, Thottappily G (1999). First report of East African cassava mosaic begomovirus in Ghana. Plant Dis. 83: 877.

Ogbe FO, Atiri GI, Robinson D, Winter S, Dixon AGO, Quin FM, Thottappily G (1999). First report of East African cassava mosaic begomovirus in Nigeria. Plant Dis. 83: 398.

Ogbe FI, Dixon AGO, Atiri GI, Thottappilly G (2002). Restriction of virus movement into axillary buds is an important aspect of resistance in cassava to African cassava mosaic virus. J. Phytopathol. 150: 546-552.

Okao-Okuja G, Legg JP, Traore L, Jorge AM (2004). Viruses Associated with Cassava Mosaic Disease in Senegal and Guinea Conakry. J. Phytopathol. 152: 69-76.

Pita JS, Fondong VN, Sangare A, Otim-Nape GW, Ogwal S, Fauquet CM (2001). Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. J. Gen Virol. 82: 655-665.

Rogers SG, Bisaro DM, Fraley RT, Hoffmann L, Band, Elmer JS, Llyod AM (1986). Tomato golden mosaic virus A component DNA in transgenic plants. Cell 45: 593-600.

Thresh JM, Fishpool LDC, Otim Nape GW, Fargette D (1994). African cassava mosaic virus disease: an underestimate and unsolved problem. Trop. Sci. 34: 3-14.

Swanson MM, Harrison BD (1994). Properties, relationships and distribution of cassava mosaic geminiviruses. Trop. Sci. 34: 15-25.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning: A laboratory Manual, Second edition, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Vanitharani R, Chellappan P, Fauquet C (2005). Geminiviruses and RNA silencing. Trends Plant Sci. 10: 1360-85.

Vanitharani R, Chellappan P, Pita J, Fauquet C (2004). Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of post- transcriptional gene silencing. J. Virol. 78: 9487-9498.

Wydra KW, Msikita W (1998). An overview of the present situation of cassava diseases in West Africa. In: Akoroda MO, Ekanayake I (eds), Root Crops and Poverty Alleviation. Proceedings of the Sixth Triennial Symposium of the International Society for Tropical Root Crops: Africa Branch, ISTRC-AB, Ibadan, Nigeria. pp. 198-206.

Zhou X, Liu Y, Calvert L, Munoz C, Otim-Nape GW, Robinson DJ, Harrison BD (1997). Evidence that DNA-A of a geminivirus associated with severe cassava disease in Uganda has arisen by interspecific recombination. J. Gen. Virol. 78: 2101-2111.

Zhou X, Robinson DJ, Harrison BD (1998). Types of variation in DNA-A among isolates of East African cassava mosaic virus from Kenya, Malawi and Tanzania. J. Gen. Virol. 79: 2835-2840.

Zhou X, Xie Y, Tao X, Zhang Z, Li Z, Fauquet CM (2003). Characterisation of DNA-β associated with begomovirus in China and evidence for co-evolution with their cognate viral DNA-A. J. Gen. Virol. 84: 237-247.