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Controlled transmission of *African cassava mosaic virus* (ACMV) by *Bemisia tabaci* from cassava (*Manihot esculenta* Crantz) to seedlings of physic nut (*Jatropha curcas* L.)

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***Jatropha curcas*, a plant with great biodiesel potential is also used to reduce the population of whiteflies, *Bemisia tabaci* on cassava fields when planted as a hedge. We therefore, investigated the transmission of *African cassava mosaic virus* (ACMV) by the whitefly vector from cassava to seedlings of 10 accessions of *J. curcas* as part of a wider investigation on the possible role of *J. curcas* as an alternative host of ACMV. Transmission tests were conducted in insect-proof cages using adult *B. tabaci* collected from ACMV-infected cassava in the field, at a rate of three adult whiteflies per *J. curcas* seedling and a transmission feeding period of four days. Twenty one (21) days after the infestation, leaf samples from individual plants of the 10 *J. curcas* accessions were tested for the presence of ACMV by the polymerase chain reaction (PCR) and the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), using the monoclonal antibodies SCRI 33. DAS-ELISA detected ACMV in five out of the ten *J. curcas* accessions while PCR detected it in eight of the 10 accessions. Furthermore, 18 out of the 35 *Nicotiana benthamiana* indicator plants mechanically inoculated with sap from symptomatic *J. curcas* seedlings produced symptoms typical of ACMV infection. This indicates that *J. curcas* is a likely host of ACMV and it may in turn, be able to infect cassava, and presumably other ACMV-susceptible hosts, in the presence of the vector.**

Key words: *Jatropha curcas* accessions, controlled transmission, mechanical inoculation, enzyme-linked immunosorbent assay, *African cassava mosaic virus* (ACMV)-susceptible hosts.

INTRODUCTION

Bemisia tabaci (Gennadius) belonging to the family Alerodidae, has been reported in all continents except Antarctica, probably due to the extremely cold conditions. The insect possibly originated in India and it is believed to have been spread throughout the world through transport of infested products (Fishpool and Burban, 1994). The insect is cosmopolitan in distribution, highly polyphagous (feeding on over 500 plant species in 74 families) and characterized by intercrop movement (Butler et al., 1986). Of the 1,100 *Bemisia* species identified, only three are

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Figure 1. A, Insect-proof cage (3 x 1.2 x 1.3 m) with *J. curcas* seedlings. **B,** clip cage containing trapped *B. tabaci* and attached to a leaf of *Jatropha* seedling to facilitate access feeding.

known to be vectors of plant viruses, with *B. tabaci* being the most important, transmitting about 111 virus species (Brown et al., 1995) including cassava mosaic Geminiviruses (CMGs) (Legg et al., 1992).

In sub-Saharan Africa, *African cassava mosaic virus* (ACMV) is probably the most economically important CMG transmitted by *B. tabaci* (Thresh et al., 1994; Geddes, 1990). The insect has also been reported to transmit *cassava brown streak virus* (CBSV, family Potyviridae) which causes cassava brown streak disease (Legg et al., 1992), and *Jatropha mosaic virus* (Brunt et al., 1996). ACMV and CBSV have been reported to cause yield losses of cassava ranging from 20 to 90% and 70%, respectively (Gration and Rey, 2012; Hillocks et al., 2001; Thresh et al., 1994). ACMV can be controlled by using healthy *in vitro* materials for planting or by a hedge crop which attracts the whitefly vector, thereby reducing their populations on cassava fields. It has been demonstrated that the populations of *B. tabaci* in cassava fields can be reduced by planting *Jatropha curcas* as a hedge crop (Ewusie et al., 2010). However, recent studies have shown that ACMV can spread from infected cassava to *J. curcas* plants in the field (Appiah et al., 2012). Additionally, Ramkat et al. (2011) have reported the co-infection of *J. curcas* by ACMV and *East African cassava mosaic virus*-Ugandan variant (EACMV-UG) in Kenya. These recent reports of the infection of *J. curcas* by ACMV and EACMV-UG render the use of *J. curcas* as a hedge crop to trap *B. tabaci* inappropriate, since virus transmission from *J. curcas* to cassava may occur. The study reported here investigated the transmission of ACMV by *B. tabaci* to seedlings of *J. curcas* under controlled conditions, to determine the degree of susceptibility and the possible role of *J. curcas* as an alternative host of ACMV.

MATERIALS AND METHODS

Vector transmission

Seeds of ten local accessions of *J. curcas* obtained from the Crop Science Department of the University of Ghana were planted in small polythene bags and arranged randomly in an insect-proof cage measuring 3 x 1.2 x 1.3 m (Figure 1). Adult *B. tabaci* were collected from ACMV-infected cassava (PCR and ELISA confirmed) in the field into small, fine-mesh clip cages which were placed directly onto the leaves of healthy *J. curcas* seedlings (Figure 2). Three adult whiteflies contained in one clip cage were placed on each *J. curcas* seedling and allowed a transmission access feeding period of four days, as described by Legg et al. (1992). The clip cages were then removed and the seedlings were sprayed with insecticide to kill the whiteflies and then observed for symptom expression over a period of 21 days. The percentage of viruliferous whiteflies, *p,* in the field collection was estimated using the formula of Gibbs and Gower (1960):

$P = 1 - (1 - R/N)\ i/1$

Where, R is the number of infected plants, N is the total number of plants tested and i is the number of whiteflies per test plant.

Virus detection

Virus detection by enzyme-linked immunosorbent assay

Monoclonal antibodies (SCRI 33) for detection of ACMV were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and used in a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described by Clark and Adams (1977). Three inoculated seedlings per *J. curcas* accession were selected at random and tested individually. An uninoculated *J. curcas* seedling and infected cassava were used as negative and positive controls, respectively. Leaf extract were prepared by grinding 1 g of young leaves in 10 ml of extraction buffer containing 20 g of polyvinylpyrrolidone (PVP), 2 g of ovalbumin, 1.3 g of sodium sulphite (anhydrous), 0.2 g of sodium

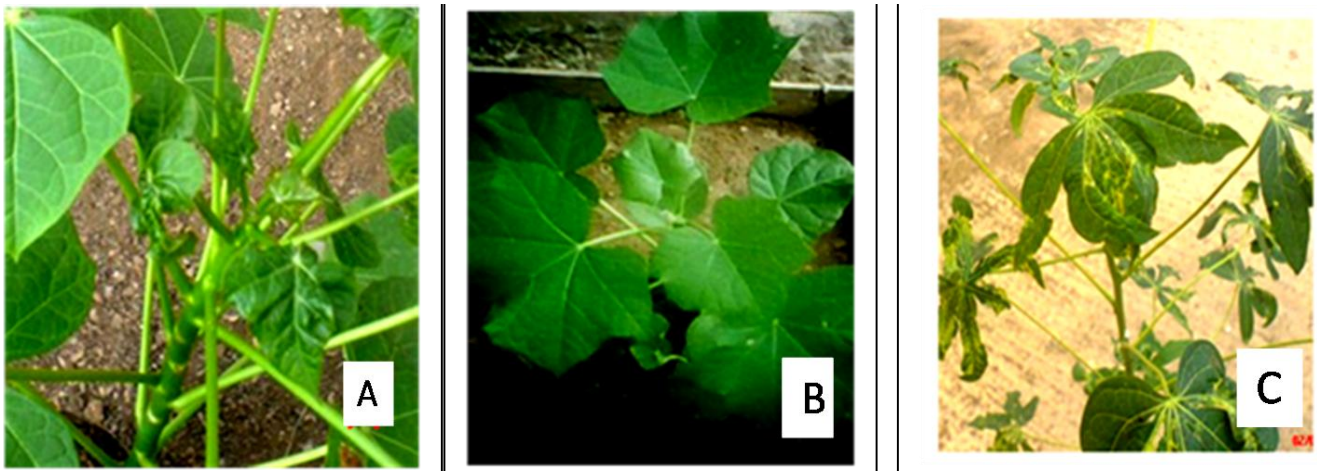

Figure 2. A, Whitefly-inoculated *J. curcas* plant showing curling and distortion of young leaves. **B,** Healthy uninoculated *J. curcas* plant, **C,** Field infected cassava showing severe symptoms of ACMD.

azide, 0.5 ml of Tween, 20.8 g of sodium chloride, 0.2 g of potassium dihydrogen orthophosphate, 1.15 g of disodium hydrogen orthophosphate and 0.2 g of potassium chloride. Absorbance values were measured at 405 nm using a spectrophotometer (Multiskan Ascent VI.25-Version 1.3.1). Samples with absorbance values of more than twice that of the negative controls were considered positive for the virus.

Virus detection by polymerase chain reaction

Total DNA was extracted from fresh leaf tissues of the *J. curcas* seedlings according to the method described by Dellaporta et al. (1983). DNA amplification was carried out with a virus-specific primer pair JSP001 (5'-ATG TCG AAG CGA CCA GGA GAT-3')/JSP002 (5'-TGT TTA TTA ATT GCC AAT ACT-3') (Fondong et al., 2000) in a 20 µl reaction mix containing 5 µl 10x reaction buffer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.25 u taq polymerase, 0.2 µM forward and reverse primers and 2 µl of template DNA. Amplification reaction was performed in a 96-well Eppendorf Thermal Cycler (Eppendorf AG, Hamburg). The PCR cycle used for the amplification consisted of an initial denaturation step at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min and elongation at 72°C for 1 min. This was then followed by a final elongation step at 72°C for 10 min. 10 µl aliquots of PCR products were mixed with 2 µl of loading dye and electrophoresed on 1% agarose gel at 90 V for 1 h. The gel was visualized under a high performance ultraviolet transilluminator (UVP, Cambridge, UK) and photographed with UVP Life Science Software (Doc - It LS Image Acquisition) and bands were scored as present or absent for analysis.

Mechanical inoculation of *Nicotiana benthamiana* indicator plants

Three-week old *N. benthamiana* indicator plants grown in an insect-proof cage were used for mechanical inoculation. The *N. benthamiana* seeds were obtained from DSMZ Plant Virus Collection, Braunschweig, Germany. Inoculum was prepared by grinding 2 g of young leaves of *J. curcas* plants in 2 ml of 0.01 M phosphate buffer pH 7.0, containing sodium sulphite in a sterilized mortar and pestle. Prior to inoculation, the *N. benthamiana* plants were pre-conditioned by topping and removing the older lower leaves, leaving four to five leaves on a seedling. The plants were then darkened by covering with black polythene sheet overnight. The leaves were dusted with carborundum powder (600 grit) followed by rubbing the inoculum gently on the leaves with the forefinger. The inoculated leaves were then gently rinsed with tap water from a wash bottle. The inoculated plants were placed under a bench overnight and then returned to the insect-proof cage and observed for symptoms up to 21 days after inoculation

Thirty five (35) *N. benthamiana* plants were inoculated with sap from symptomatic *J. curcas* seedlings, while 18 were inoculated with sap from symptomless *J. curcas* seedlings. Samples were collected from inoculated symptomatic plants as well as symptomless ones for ELISA detection as described previously.

RESULTS AND DISCUSSION

ACMV transmission by *B. tabaci* and symptom development in *J. curcas*

Adult *B. tabaci* collected from ACMV-infected cassava in the field transmitted ACMV to seedlings of all ten accessions of *J. curcas*. Symptoms of leaf curling, leaf distortion and reduction in size of leaf blades, mostly of young leaves (Figure 2), were observed 21 days after the transmission feeding period. Similar symptoms have been reported in other crops as the most frequently associated with Begomovirus infection (Seal et al., 2006; Wang et al., 2004). The mosaic symptoms that normally characterizes ACMV infection of cassava (Figure 2C) were however, not evident on the infected *J. curcas* plants. The absence of this usual mosaic symptom on young *J. curcas* may be attributed to differences in the texture or the anatomy of the leaf lamina.

ELISA detection of ACMV in *J. curcas*

The double-antibody sandwich enzyme-linked immuno-

Table 1. DAS-ELISA detection of *African cassava mosaic virus* in leaf extracts of ten accessions of *J. curcas* seedlings inoculated by adult *Bemisia tabaci*.

Accession	plant number	Absorbance (405 nm) (mean ± SE)	ELISA Reaction (+ or -)
	1	0.131 ± 0.0008	-
Addogon	2	0.466 ± 0.0034	+
	3	0.542 ± 0.0004	+
	1	0.360 ± 0.0014	-
Aklamado	2	0.215 ± 0.0004	-
	3	0.319 ± 0.0010	-
	1	0.277 ± 0.0122	-
Amanfrom	2	0.169 ± 0.0007	-
	3	1.006 ± 0.0010	+
	1	0.328 ± 0.0004	-
Apeguso	2	0.376 ± 0.0004	-
	3	0.277 ± 0.0118	-
	1	0.397 ± 0.0018	+
Asamankese	2	0.351 ± 0.0009	-
	3	0.429 ± 0.0008	+
	1	0.065 ± 0.0004	-
Gbefi	2	0.068 ± 0.0008	-
	3	0.250 ± 0.0004	-
	1	0.244 ± 0.0008	-
Hohoe	2	0.101 ± 0.0005	-
	3	0.440 ± 0.0008	+
	1	0.077 ± 0.0004	-
Kasoa	2	0.191 ± 0.0010	-
	3	0.539 ± 0.0008	+
	1	0.268 ± 0.0004	-
Kpeve	2	0.339 ± 0.0018	-
	3	0.144 ± 0.0008	-
	1	0.239 ± 0.0008	-
Valley View University	2	0.156 ± 0.0012	-
	3	0.095 ± 0.0020	-
Uninoculated *J. curcas* seedling (negative control)		0.197 ± 0.0021	-
ACMV-infected cassava (positive control)		1.449 ± 0.0021	+

Values are means of four tests; absorbance values more than twice that of the negative control were considered positive for the virus. + = ACMV positive; - = ACMV negative.

sorbent assay (DAS-ELISA) detected ACMV in leaf extracts of the *J. curcas* seedlings inoculated with *B. tabaci* (Table 1). Of the 30 seedlings tested (three per accession), seven from five accessions had UV absorbance values twice that of ACMV-free, indicating that they were infected by the virus. The accession

Figure 3a. Testing of *J. curcas* seedlings before whitefly inoculation by PCR using ACMV-specific primer pair, JSP001/JSP002. Lane 1 = 1 kb DNA ladder; 2 = Hohoe; 3 = Kasoa; 4 = Apeguso; 5 = Kpeve; 6 = Addogon; 7 = Valley View University; 8 = Asamankese; 9 = Gbefi; 10 = Aklamado; 11 = ACMV-infected cassava (positive control); 12 = Amanfro; 13 = water control; 14 = positive control.

Figure 3b. Testing of *J. curcas* plants 21 days after whitefly inoculation by PCR using ACMV-specific primer pair, JSP001/JSP002. Lane 1, 1 kb DNA ladder; 2, Hohoe; 3, Kasoa; 4, Apeguso; 5, Kpeve; 6, Addogon; 7, Valley View University; 8, Asamankese; 9, Gbefi; 10, Aklamado; 11, Amanfro; 12 and 13, positive control; 14, uninoculated *J. curcas* and 15, water control.

Amanfrom, gave the highest positive absorbance of 1.006 whilst the least (0.397) was from Asamankese accession.

With three adult whiteflies per *J. curcas* seedling, an ACMV transmission of 23% was achieved.Our observation is comparable with that of Rashid et al. (2008) who reported 20% transmission for tomato yellow leaf curl virus (TYLCV) with three infective adult whiteflies per tomato seedling.

The low virus transmission recorded in this study and that of Rashid et al. (2008) could be attributed to the relatively low numbers of adult whiteflies per plant. Although, a single whitefly has been shown to transmit ACMV, the percent transmission increased with increasing number of whiteflies per plant (Mahto and Sinha, 1978).

Mathew (1988) achieved 100% transmit-ssion of Indian cassava mosaic virus to *N. benthamiana* with over 40 adult whiteflies per plant. Similarly, Nguessan et al. (1992) achieved 50% transmission of okra leaf curl virus (OLCV) when each plant was infested with 25 adult whiteflies, even though the estimated percentage of viruliferous whiteflies was 4% as compared to the 8.5% recorded in this study.

Polymerase chain reaction detection of ACMV in *J. curcas*

The polymerase chain reaction detected ACMV in all the *J. curcas* accessions except Gbefi and Apeguso (Figure 3). The technique was able to detect the virus in accessions Valley View University, Aklamado and Kpeve that tested negative to DAS-ELISA. This could be due to the enhanced sensitivity of the PCR technique (Martin, 1998) as compared to ELISA, making it the preferred method for the detection and characterization of viral infections that are difficult to detect and diagnose by serology (Seal and Coates, 1998). Disparities in the detection of ACMV by ELISA and PCR have been reported by Okorogri et al. (2010).

The primer pair JSP001/JSP002 amplified the expected products of size ~0.7 kb in the coat protein gene of the virus. Adjata et al. (2008a), Mbasa (2007) and Pita et al. (2001) have independently used this same primer for successful detection of ACMV in cassava.

Figure 4. A, Uninoculated *N. benthamiana* test plant. **B,** Inoculated *N. benthamiana* test plant showing leaf yellowing, leaf distortion, reduced leaf size and stunting 14 days after inoculation.

Response of *N. benthamiana* indicator plants to mechanical sap inoculation

Of 35 *N. benthamiana* indicator plants inoculated with sap from symptomatic *J. curcas* seedlings, 18 (51.4%) produced symptoms characteristic of ACMV infection. None of the *N. benthamiana* test plants inoculated with sap from healthy *J. curcas* seedlings expressed symptoms.

Symptoms observed on the *N. benthamiana* indicator plants were leaf yellowing and distortion, reduced leaf size and stunting (Figure 4). These symptoms were similar to those produced in test plants inoculated with sap from ACMV-infected cassava (positive control). Sixteen (16) out of the 30 (53.3%) inoculated *N. benthamiana* plants tested by DAS-ELISA were successfully infected with the virus. Furthermore, 20% of the test plants that did not visually express symptoms following the sap inoculation also tested positive for the virus by DAS-ELISA (Table 2). Symptomless infection of sap-inoculated test plants has been reported by Hemida (2005) and Dijkstra et al. (1996). Some of the indicator plants inoculated with either PCR or ELISA-negative samples of accessions Aklamado, Apeguso and Kpeve were symptomatic. Although, the cassava source plants from which the whiteflies were collected were indexed for ACMV, the possibility of mixed infection with other CMGs could not be ruled out. The disparities in the results obtained by the different detection techniques in *J. curcas* seedlings and *N. benthamiana* indicator plants may be due to mixed infection of the donor material by CMGs, a situation which has been reported in Ghana by Were et al. (2003).

The results of this study demonstrate that *J. curcas* is a possible host of ACMV which may serve as a source of virus infection to healthy cassava and, presumably, other ACMV-susceptible hosts when the whitefly vector is present. Further investigations using ACMV-infected *J. curcas* as a source of virus to infect cassava are needed to confirm that *J. curcas* is indeed an alternative host of ACMV. The plant should also be evaluated for other cassava mosaic geminiviruses reported in West Africa such as the EACMV (Offei et al., 1999; Were et al., 2003) and the *Indian cassava mosaic virus* (Adjata et al., 2008b).

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Table 2. DAS-ELISA detection of ACMV in *N. benthamiana* test plants inoculated with sap from *J. curcas* seedlings.

***J. curcas* accession: source of inoculum**	***N. benthamiana* test plant**	**Symptom expression on inoculated *N. benthamiana***	**ELISA reaction**
	1	No	-
Addogon	2	Yes	+
	3	Yes	+
	1	Yes	-
Aklamado	2	No	-
	3	Yes	+
	1	No	-
Amanfrom	2	No	+
	3	Yes	+
	1	Yes	+
Apeguso	2	No	+
	3	Yes	+
	1	Yes	+
Asamankese	2	Yes	+
	3	No	-
	1	No	+
Gbefi	2	No	-
	3	No	-
	1	Yes	+
Hohoe	2	Yes	-
	3	No	-
	1	Yes	+
Kasoa	2	No	-
	3	Yes	+
	1	Yes	+
Kpeve	2	Yes	+
	3	No	-
	1	No	-
Valley View Univ.	2	No	-
	3	No	-

+**,** ACMV positive; -, ACMV negative.

REFERENCES

Adjata KD, Muller E, Aziadekey M, Gumedzoe YMD and Peterschmitt M (2008b). Incidence of Cassava Viral Diseases and First Identification of East African cassava mosaic virus and Indian cassava mosaic virus by PCR in Cassava (Manihot esculenta Crantz) Fields in Togo. Amer. J. Plant Physiol. 3:73-80.

Adjata KD, Muller E, Peterschmitt OT, Crumedzoe YMD (2008a). Molecular evidence for the Association of a strain of Uganda variant of East African cassava mosaic virus to symptom severity in cassava (*Manihot esculenta* Crantz) fields in Togo. Am. J. Biochem. Biotechnol. 5 (4):196-201).

Appiah AS, Amoatey HM, Klu GYP, Afful NT, Azu E, Owusu GK (2012). Spread of African cassava mosaic virus from cassava (*Manihot esculenta* Crantz) to physic nut (*Jatropha curcas* L.) in Ghana. J.Phytol. 3(12):35-40.

Brown JK, Frohlich DR, Rosell RC (1995). The sweet potato silver leaf whiteflies: Biotypes of *B. tabaci,* a species complex? Annu. Rev.

Entomol. 40:511-534.
Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (Eds) (1996 onwards). Plant viruses online: Descriptions and lists from VIDE Database.Version: 20th August, 1996 URL. http://biology.anu.edu.au/Groups/MES/vide/
Butler GD Jr., Henneberry TJ, Hutchinson WD (1986). Biology, sampling and population dynamics of *Bemisia tabaci*. Agric. Zool. Rev. 1:167-195.
Clark MF, Adams AN (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
Dellaporta S, Wood L, Hicks JB (1983). A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1(14):19-21.
Dijkstra J, Keesen E, Brink M, Peters D, Lohuis H (1996). Identification and characterisation of a potyvirus of Bambara groundnut. Afr. Crop Sci. J. 4(1):97-104.
Ewusie EA, Parajulee MN, Aba Adabie-Gomez D and Wester D (2010). Strip Cropping: A Potential IPM Tool for Reducing Whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) Infestations in Cassava. W. Afr. J. Appl. Ecol. 17:109-119.
Fishpool LDC, Burban C (1994). *Bemisia tabaci*: the whitefly vector of African cassava mosaic geminivirus. Trop. Sci. 34 (1):55-72.
Fondong VN, Pita JS, Rey MEC, de Kochko A, 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.
Geddes AMW (1990). The relative importance of crop pests in sub-Saharan Africa. NRI Bulletin No. 36, Chatham, U.K.
Gibbs AJ, Gower J-C (1960). The use of multiple transfer method in plant virus transmission studies - some statistical points arising in the analysis of results. Ann. Appl. Biol. 48:75-83.
Gration MR, Chrissie MER (2012). Relationship between Symptoms Expression and Virus Detection in Cassava Brown Virus Streak-Infected Plants. J. Agric. Sci. 4(7):246-253.
Hemida SK (2005). Effect of bean yellow mosaic virus on physiological parameters of *Vicia faba* and *Phaseolus vulgaris*. Int. J Agric. Biol. 7 (2):154-157.
Hillocks RJ, Raya MD, Mtunda K, Kiozia H (2001). Effects of cassava brown streak virus disease on yield and quality of cassava in Tanzania. J. Phytopath. 149:389-394.
Legg JP, Asiimwe P, Sseruwagi P, Brown JK (1992). Changes in the biology of *Bemisia tabaci* on cassava in Africa and their impact on virus disease pandemics. Fourth International Bemisia Workshop: International Whitefly Genomics Workshop. J. Insect Sci. (8):4. http://www.insectscience.org/8.04/ref/abstract51.html
Mahto DN, Sinha DC (1978). Mosaic disease of cassava and its relationship with the vector, Bemisia tabaci Genn. Indian J. Entomol. 40:117-120
Martin RR (1998). Advanced Diagnostic Tools as an Aid to Controlling Plant Virus Disease. In: Plant virus disease control. Hadidi, A, Khetarpal, RK, Koganezawa, H (Eds). American Phytopathological Society, Minnesota, USA. pp. 399-416.
Mathew AV (1988). Studies on Indian cassava mosaic virus disease. PhD Thesis, University of Agricultural Science, Bangalore, India. pp. 237
Mbasa KG (2007). Epidemiology of cassava mosaic disease and molecular characterization of cassava mosaic viruses and their associated whitefly (*Bemisia tabaci*) vector in South Africa. M Sc. Thesis. University of the Witwatersrand, Johannesberg. South Africa. pp. 34-35.
Nguessan KP, Fargette D, and Fauquet C, Thouvenel JC (1992). Aspects of epidemiology of okra leaf curl virus in Cote d'Ivoire. Trop. Pest Manage. 38(2):122-126.
Offei SK, Owuna-Kwakye M, Thottappilly G (1999). First report of East African cassava mosaic begomovirus in Ghana. Plant Dis. 83 (9):877.
Okorogri EB, Adetimirin VO, Ssemakula G, Odu B, Dixon AGO (2010). Rate of re-infection of tissue culture -derived cassava genotypes by mosaic disease. Afr. J. Biotechnol. 9(51):8748-8753.
Pita JS, Fondong VN, Sangara 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.
Ramkat RC, Calari A, Maghuly F, Laimer M (2011). Biotechnological approaches to determine the impact of viruses in the energy crop plant *Jatropha curcas*. Virol. J. 8:386.
Rashid MH, Hossain I, Alam MS, Zaman MM, Hannan A (2008). Study of virus-vector relationship in TYLC of Tomato. Int. J. Sustain. Crop Prod. 3(1):1-6.
Seal S, Coates D (1998). Detection and Quantification of Plant viruses by PCR. In: Plant Virology Protocols. Forster, GD and Taylor, SC (Eds.). Vol. (81)469-485.
Seal SE, van den Bosch, F and Jeger, MJ (2006). Factors influencing Begomovirus evolution and their increasing global significance: implications for sustainable control. Crit. Rev. Plant Sci. 25: 23-46.
Thresh JM, Fishpool DC, Otim-Nape GW, Fargette D (1994). African Cassava Mosaic Virus: an underestimated and unresolved problem. Trop. Sci. 34:3-14.
Wang XY, Xie Y, Zhou XP (2004). Molecular characterization of two distinct begomoviruses from papaya in China. Virus Genes 29:303-309.
Were HK, Winter S, Maiss E (2003). Distribution of Begomoviruses infecting cassava in Africa. J. Plant Pathol. 85(3):145-151.