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Spread of African cassava mosaic virus from cassava (*Manihot esculenta* Crantz) to physic nut (*Jatropha curcas* L.) in Ghana

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Abstract
Investigations were made on the susceptibility of physic nut (*Jatropha curcas* L.) to African cassava mosaic virus (ACMV) and its possible role as an alternative host of the virus in Ghana. Ten *J. curcas* accessions in a field trial were interplanted with ACMV-infected cassava (*Manihot esculenta* Crantz) and left to natural spread of the virus from the cassava to the *J. curcas* plants for a period of 12 months. Populations of the whitefly vector, *Bemisia tabaci* (Gennadius) and the incidence of African cassava mosaic disease (ACMD) were monitored during the period. The *J. curcas* plants had low whitefly numbers, both in the wet (September – October, 2008) season and in the dry (January – February, 2009) season. By the end of the experimental period, 37.7% of the 120 *J. curcas* plants tested in all the accessions were found to be infected by ACMV, as assessed by symptom expression, double antibody-sandwich (DAS) ELISA or sap inoculation to *Nicotiana benthamiana* indicator plants. There were wide variations within and between the *J. curcas* accessions in their response to ACMV infection. This work is the first report of the natural infection of *J. curcas* by ACMV.

Keywords: African cassava mosaic virus, symptom severity, enzyme-linked immunosorbent assay, indicator plants, *Jatropha curcas* accessions, *Nicotiana benthamiana*.

INTRODUCTION

Physic nut (*Jatropha curcas* L.) is a drought-resistant shrub or small tree belonging to the family Euphorbiaceae and producing oil-containing seeds [1]. Recently, there has been great interest in the cultivation of physic nut for biodiesel production, especially since it is not grazed by animals, grows readily on marginal soils, is drought resistant, multi-purpose and yields high quality biodiesel [2] This has created a great deal of attention, resulting in the planting of large plantations in Asia, Africa and the Americas.

There are claims that physic nut possesses many other useful qualities, such as tolerance to diseases and pests. This is based on mere observation of solitary plants, and does not apply in general to physic nut grown in plantations. Large-scale cultivation of physic nut, especially under humid conditions, encounters serious problems including fungal, viral and insect attacks [3]. [4] reported that *J. curcas* could harbour cassava superlongation disease (*Sphaceloma manihoti* / *Elsinoe brasiliensis*), while *Jatropha multifida* was an alternative host of African cassava mosaic virus (ACMV); it was, therefore, assumed that this may also apply to *J. curcas*. The study reported here was to address the knowledge gap of the possible role of *Jatropha curcas* as an alternative host of ACMV in Ghana.

MATERIALS AND METHODS

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Plant material and field design

Ten *Jatropha curcas* accessions were used for the study. The accessions had been previously selected from different areas of southern Ghana and assembled at the Botanical Garden of the University of Ghana. The field treatments were assigned in a randomized complete block design (RCBD) replicated four times. Ten (10) plants of each *J. curcas* accession were planted in a row at a distance of 1.0m within rows and 1.5m between rows and interplanted with ACMV-infected cassava cultivar *Afisiafi*, also at 1.0m within a row. The *J. curcas* stock plants from which cuttings were taken for the field planting were indexed by DAS-ELISA as described by [5] and found to be free from ACMV.

The cassava cultivar *Afisiafi* used for the interplanting is high yielding and widely grown in Ghana, but is highly susceptible to ACMV. It was considered that it would serve as a good source of ACMV to the *J. curcas* test plants. All the cassava plants which provided cuttings for the field planting were indexed by DAS-ELISA and confirmed to be ACMV-infected.

Fig 1. Field layout showing *Jatropha curcas* interplanted with ACMV-infected cassava cultivar *Afisiafi* (middle row)

Whitefly survey

Five plants of each *J. curcas* accession were tagged and individually monitored for whitefly populations. The assessment was done by counting the number of adult whiteflies on the five topmost leaves of each plant [6]. Insect count was started at eight weeks after planting (WAP) when the plants had developed sufficient foliage to support adult whitefly populations. Counting continued over a 2-month period at weekly intervals, from September to October, 2008 (wet season) and from January to February, 2009 (dry season).

Scoring and estimating for symptom severity and disease incidence

Leaf symptoms on the *J. curcas* plants were scored on a five-point scale [7]. Scoring was started when the plants were eight weeks old and continued monthly for three months. The scoring scale was as follows

1. No symptoms
2. Mild blistering over all or part of the leaf, curling and distortion in one or two of the five topmost leaves.
3. Moderate blistering or puckering throughout the leaf, curling and distortion in three of the five topmost leaves.
4. Severe blistering or puckering, curling and general distortion in four of the five topmost leaves.
5. Severe blistering or puckering and distortion of all the five topmost leaves.

Index of severity of symptoms based on all plants (ISS_{AP}), Index of severity of symptoms based on diseased plants only (ISS_{DP}), as well as disease incidence (DI%) were estimated according to [8].

Detection of ACMV by enzyme-linked immunosorbent assay (ELISA)

A panel of monoclonal antibodies (SCRI 33) which detects African cassava mosaic virus (ACMV) was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and used in a direct double-antibody sandwich ELISA (DAS-ELISA) according to [5]. Leaf samples from twelve symptomatic plants of each *J. curcas* accession were tested at six, nine and twelve months after planting.

Mechanical inoculation of *Nicotiana benthamiana*

Mechanical inoculation was carried out on three-week old *Nicotiana benthamiana* indicator plants raised in an insect-proof cage located in a plant barn. The *N. benthamiana* seeds were obtained from a commercial dealer, DSMZ Plant Virus Collection, Braunschweig, Germany. The inoculum was prepared by grinding 2g of young leaves from the *J.curcas* or the cassava (control) plants in 2 ml of 0.01 M phosphate buffer pH 7.0, containing sodium sulphite, in a sterilized mortar and pestle.

The *N. benthamiana* plants were prepared for inoculation by topping and removing the older lower leaves and giving a pre-inoculation darkening overnight. The leaves were then dusted with carborundum powder (600 grit) and rubbed gently with the forefinger wet in the inoculum. The inoculated leaves were given a gentle rinse with tap water [9]. The plants were placed under a bench overnight and then returned to the insect-proof cage and observed for symptoms. After four weeks of observation, inoculated plants which did not show symptoms were further tested by DAS-ELISA as previously described.

Data analysis

Data on whitefly numbers and symptom scores for diseased plants as well as for all plants were subjected to square root transformation before analysis of variance (ANOVA). ACMV incidence was obtained by making counts of infected samples from DAS-ELISA and converting them to percentages of the total number of samples per *Jatropha* accession. Disease incidence percentages were arcsine transformed and the transformed values subjected to analysis of variance. All data were analyzed using Genstat statistical software, 12th Edition. Treatment means were compared using the least significance difference test.

RESULTS

Symptoms of ACMV disease on *Jatropha curcas* plants interplanted with ACMV-infected cassava

ACMV disease symptoms observed on the *J. curcas* plants were leaf curling and distortion, blistering on leaf surfaces and severe reduction of the leaf blade especially in young leaves (Fig. 1). Similar symptoms were observed on the *Nicotiana benthamiana* indicator plants inoculated with sap from ACMV-infected *J. curcas*. However, the characteristic leaf mosaic symptom associated with African cassava mosaic disease (ACMD) on cassava was not evident.

A B

Fig. 2. A: *J. curcas* plant showing symptoms of ACMV disease. Note the leaf curling, leaf distortion, blistering on leaf surfaces and reduction of leaf size. B: Healthy *J. curcas* plant with no disease symptoms

The results of assessment of the incidence of ACMV disease on the *J. curcas* plants are shown in Table 1. At eight weeks after planting (WAP), plants from five out of the ten accessions were symptomless and some plants of the other five accessions expressed only mild symptoms, DI: 5% and ISS_{DP}: 2.0, which were not significantly different ($p > 0.05$) from those which showed no symptoms.

At 12 WAP, all the accessions except Kpeve, had plants which showed symptoms of ACMV infection. There were no significant differences ($p > 0.05$) in terms of disease incidence, ISS_{AP} and ISS_{DP} among the accessions. The highest disease incidence (30.0%) was recorded on plants of accession Hohoe which also had the highest ISS_{AP} of 1.30. However, accession Gbefi, which had a disease incidence of 10%, recorded the highest ISS_{DP} of 2.5.

At 16 WAP, all the accessions had plants which showed clear symptoms of ACMV disease, but there were degrees of symptom expression. The highest disease incidence of 45.0% was recorded in accessions Amanfro, Asamankese, Hohoe and Kasoa, although this was not significantly different ($p > 0.05$) from incidence in the rest of the accessions. Accession Addogon recorded the highest ISS_{AP} and ISS_{DP} of 1.75 and 3.00 respectively. The lowest disease incidence (35.0%) was recorded in accessions Addogon and Valley View University.

Table1. Incidence of ACMV disease and symptom severity on plants of ten local accessions of *Jatropha curcas* intercropped with ACMV-infected cassava.

Weeks after Planting (WAP)		*J. curcas* accessions									
		Add	**Akl**	**Ama**	**Ape**	**Ask**	**Gbe**	**Hoe**	**Kas**	**Kpe**	**VVU**
8	DI (%)	0	5.0	0	5.0	0	5.0	5.0	0	0	5.0
	ISS_{AP}	1.00	1.05	1.00	1.05	1.00	1.05	1.05	1.00	1.00	1.05
	ISS_{DP}	nil	2.0	nil	2.0	nil	2.0	2.0	nil	nil	2.0
12	DI (%)	20.0	10.0	10.0	15.0	5.0	10.0	30.0	10.0	0.00	20.0
	ISS_{AP}	1.30	1.10	1.10	1.15	1.05	1.15	1.30	1.10	1.00	1.20
	ISS_{DP}	2.0	2.0	2.0	2.0	2.0	2.5	2.0	2.0	nil	2
16	DI (%)	35.0	40.0	45.0	35.0	45.0	40.0	45.0	45.0	40.0	35.0
	ISS_{AP}	1.75	1.55	1.65	1.60	1.65	1.55	1.75	1.65	1.50	1.55
	ISS_{DP}	3.00	2.38	2.50	2.62	2.45	2.38	2.75	2.50	2.25	2.50

There were no significant differences ($p > 0.05$) among the accessions in respect of DI (%), ISS_{AP} and ISS_{DP}
DI (%) = percent disease incidence
ISS_{AP} = index of severity of symptoms on all plants
ISS_{DP} = index of severity of symptoms on diseased plants only
J. curcas accessions
Add = Addogon; Akl = Aklamado; Ama = Amanfro; Ape = Apeguso; Ask = Asamankese;
Gbe = Gbefi; Hoe = Hohoe; Kas = Kasoa, Kpe = Kpeve and VVU = Valley View University.

Assessment of whitefly populations on the ten *Jatropha curcas* accessions

Results of the assessment of whitefly populations on the *J. curcas* plants are shown in Figure 2. Mean whitefly numbers recorded on all the *Jatropha* accessions were relatively lower in the wet season (September – October, 2008) than in the dry season (January – February, 2009). In the wet season, there were no significant differences ($p > 0.05$) among the mean numbers of whiteflies found on the *Jatropha* accessions, but the highest mean number of adult whiteflies (0.9) was found on accession Apeguso whilst accessions Gbefi and Amanfro had the least mean number (0.3).

In the dry season, however, there were significant differences ($p < 0.05$) among the mean numbers of adult whiteflies recorded on the accessions. The highest mean number (2.8) was recorded on accession Asamankese and this was significantly different from the numbers recorded on accessions Apeguso (0.5), Amanfrom (1.0), Hohoe (0.8), Kasoa (1.6) and Valley View University (1.2).

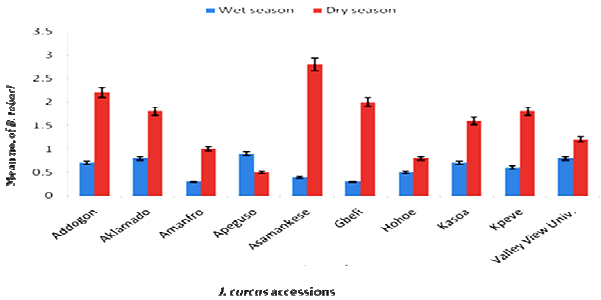

Fig 3. Mean numbers of *Bemisia tabaci* recorded on the ten *J. curcas* accessions.Vertical bars indicate standard errors

Detection of ACMV by DAS-ELISA in leaf extracts of the ten *Jatropha curcas* accessions

The ACMV infection status of the ten *J. curcas* accessions as determined by DAS-ELISA at six, nine and twelve months after planting (MAP) is shown in Fig. 3. At six MAP, there was no indication of infection in plants of accessions Aklamado, Amanfro, Asamankese and Gbefi but the remaining accessions tested positive. The highest ACMV infection (16.7%) was in plants of accessions Kasoa and Kpeve but this was not significantly different ($p > 0.05$) from the infection recorded in accessions Addogon, Apeguso, Hohoe and Valley View University.

At nine MAP, all accessions except Aklamado were infected but the percent infection showed no significant differences ($p > 0.05$) among the accessions. The highest infection (25.0%) was in accession Apeguso.

All accessions showed ACMV infection at 12 MAP. The highest rate of infection (50.0%), which was not significantly different ($p > 0.05$) from infection in the other accessions, was recorded in accession Aklamado which gave no indication of infection at six and nine MAP, and in accession Kasoa. In general, the rate of infection of the accessions increased with increasing MAP

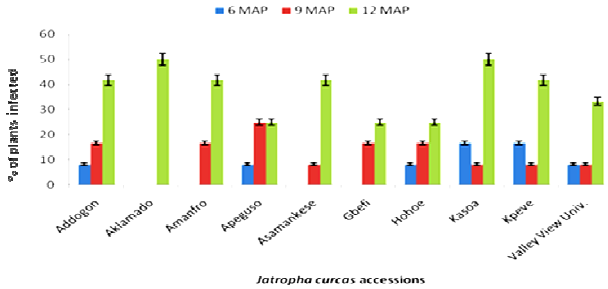

Fig 4. Percent ACMV infection among plants in the ten *J. curcas* accessions as detected by DAS-ELISA at six (6), nine (9) and twelve (12) months after planting (MAP).Vertical bars represent standard errors.

Use of *Nicotiana benthamiana* to test for ACMV infection of *Jatropha curcas*

The results obtained following mechanical inoculation of *N. benthamiana* test plants with sap from *J. curcas* are shown in Table 1. Due to shortage of test plants, only 30 out of the 120 *J. curcas* plants in which the virus was detected by DAS-ELISA at 12 MAP were tested, and included three plants from each accession. Five *N. benthamiana* test plants were inoculated per *J. curcas* plant. Only three test plants inoculated with sap from accessions Asamankese, Aklamado and Kpeve produced characteristic systemic symptoms 11 – 14 days after inoculation. The dominant symptoms were systemic leaf curling and reduced leaf size (Fig. 4) which were similar to those produced in test plants inoculated with sap from ACMV-infected cassava (positive control). Test plants inoculated with sap from healthy *J. curcas* plants, however, did not produce any symptoms. Some of the test plants inoculated with sap from accessions Addogon, Hohoe and Kasoa wilted and died from unknown cause (Table 3).

Inoculated *N. benthamiana* test plants that failed to produce symptoms were selected at random and subjected to DAS-ELISA. The results showed that of the seventeen *N. benthamiana* plants tested, ACMV was present in eleven of them, which should also apply to the *J. curcas* plants from which they were inoculated (Table 3). The highest positive absorbance value (2.710 ± 0.049) was recorded in the test plant inoculated with sap from Addogon plant number 6 and the least was 1.184 ± 0.033 from Hohoe plant number 3. Uninoculated *N. benthamiana* (negative control) gave an absorbance value of 0.524 ± 0.019 whilst ACMV-infected cassava (positive control) gave an absorbance value of 2.726 ± 0.010 that was about 1.678 higher than the threshold.

Table 2. Mechanical inoculation of *Nicotiana benthamiana* to test for ACMV infection in *Jatropha curcas*

Source of inoculum	No. of *N. benthamiana* plants inoculated	No. of inoculated plants that showed symptoms	No. of inoculated plants that died
Addogon	15	0	1
Aklamado	15	1	0
Amanfrom	15	0	0
Apeguso	15	0	0
Asamankese	15	1	0
Gbefi	15	0	0
Hohoe	15	0	3
Kasoa	15	0	2
Kpeve	15	1	0
Valley View University	15	0	0
Infected cassava (positive control)	15	8	0
Healthy *J. curcas* (Negative control)	15	0	0

Fig 5. A: *N. benthamiana* test plant showing systemic leaf curling and distortion 21 days after mechanical inoculation with *J. curcas* sap. B: Healthy uninoculated *N. benthamiana.*

Table 3. ELISA detection of ACMV in leaf extracts of *Nicotiana benthamiana* test plants following mechanical inoculation with *Jatropha curcas* sap.

Source of inoculum	Absorbance (405 nm) Mean ± SE	ELISA reaction (+ or -)
Add 6	2.710 ± 0.049	+
Add 5	1.530 ± 0.019	+
Akl 6	1.443 ± 0.065	+
Ama 4	0.867 ± 0.007	-
Ama 6	1.249 ± 0.067	+
Ape 4	1.815 ± 0.019	+
Ape 5	0.941 ± 0.020	-
Ask 4	1.223 ± 0.029	+
Gbe 3	1.242 ± 0.093	+
Gbe 5	1.224 ± 0.016	+
Hoe 3	1.184 ± 0.033	+
Kas 6	0.977 ± 0.095	-
Kas 1	1.435 ± 0.019	+
Kas 4	1.489 ± 0.063	+
Kpe 5	0.902 ± 0.029	-
VVU 1	0.913 ± 0.030	-
VVU 3	1.045 ± 0.018	-
ACMV-free*N.benthamiana* (negative control)	0.524 ± 0.019	-
ACMV-infected cassava (positive control)	2.726 ± 0.010	+
Threshold (twice negative control)	1.048	

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

DISCUSSION

Whitefly populations and ACMV disease symptom expression on the ten *Jatropha curcas* accessions

The study has shown variation in whitefly populations on plants of the *J. curcas* accessions at different times of the year. That whitefly populations were higher during the dry season than in the wet season may be due to the suppression of oviposition and the high mortality of first instar larvae associated with high rainfall during the wet season, as suggested by [10]. Other researchers working on cassava [11] and on cotton [12] also observed a reduction in whitefly populations in the wet season which they attributed to higher rainfall. In contrast, [13] recorded the highest population of adult whiteflies during high rainfall months and attributed it to the development of flushes of new leaves which were preferred by the whiteflies. Whitefly populations recorded on the *J. curcas* plants were generally low as compared to the populations recorded on cassava in the present study and as reported by [14]. This suggests that the whiteflies prefer cassava to *Jatropha* as a host plant.

The study has shown considerable variation in ACMV disease symptom expression among plants of the ten *J. curcas* accessions with time. The mild symptoms on the plants at eight weeks after planting (WAP) may be attributed to low numbers of whiteflies on the plants at this early stage of their development. It may also be a reflection of the time interval between vector feeding and disease development.

Symptom development in the *J. curcas* plants, however, increased progressively at 12 and 16 WAP, culminating in increased disease incidence. The increase in disease incidence could be attributed to the increased numbers of whiteflies then found on the plants. This agrees with the results of [15] in which higher whitefly numbers per plant in different agro-ecological zones resulted in higher ACMV disease incidences in cassava varieties. Positive correlations have also been observed between *B. tabaci* populations and cassava mosaic disease (CMD) spread into initially healthy cassava plantings [16]. These observations suggest that disease spread might be facilitated by a high population density of *B. tabaci.* Thus, the *J. curcas* accession Asamankese which recorded the highest mean number of whiteflies (2.8) also gave a high disease incidence value of 45.0%. This was, however, not always the case as accessions Amanfrom, Kasoa, and Hohoe had high values of disease incidence (45.0%) at 16 WAP, similar to that of accession Asamankese, but low mean numbers of whiteflies. This may suggest that symptom expression among the accessions may have been influenced by other yet unknown factors.

Detection of African cassava mosaic virus in plants of ten accessions of *Jatropha curcas* by DAS-ELISA

The detection by DAS-ELISA of ACMV in plants of all the ten *J. curcas* accessions provides a proof for the assumption by [4] that *J. curcas* may be a host of ACMV. The detection however, varied greatly with age of plants. This is in agreement with work on cassava mosaic disease (CMD) in Uganda in which CMD incidence increased with MAP in most of the cassava accessions tested [17]. A similar observation was made on tomato leaf curl virus (ToLCV) in which disease incidence increased dramatically with weeks after planting [18]. The increase in ACMV infection of the *J. curcas* plants with time in this study may be due to the continuous exposure of the plants to the source of inoculum (infected cassava), which has been shown to be very important in the epidemiology of cassava mosaic disease (CMD) [19].

The decreased detection of ACMV in the *J. curcas* accessions Kasoa and Kpeve at nine MAP may be due to the uneven systemic spread that characterizes virus infection of woody plants. Most canopies of virus-infected woody plants may be symptomless and the virus is almost undetected in these organs. *J curcas* accession

Aklamado in which the virus could not be detected at six and nine MAP, was found to be the most infected at 12 MAP, with about 50% of the test plants infected. It is likely that plants of this accession may have merely escaped infection in the early months after planting, as reported for cassava [7]. The delay in virus detection in plants of this accession could also be due to low virus concentration in the early stages of the experiment. However, the high rate of ACMV detection at 12 MAP suggests that the virus multiplied rapidly after the early stages, reaching a concentration sufficient for its detection.

Nicotiana benthamiana as a test plant for the detection of ACMV in *Jatropha curcas*

This study has clearly demonstrated the transmission of ACMV from *J. curcas* to *N. benthamiana* by mechanical inoculation with infective sap. However, only three *N.benthamiana* test plants (about 2% of the total), inoculated with sap from plants of accessions Aklamado, Asamankese and Kpeve produced characteristic symptoms as reported by [20].

Nonetheless, ACMV was readily detected by DAS-ELISA in eleven samples from the inoculated *N. benthamiana* test plants which failed to produce symptoms. This observation is in agreement with that reported by [21] in which mechanical sap inoculation of bean yellow mosaic virus to *Pisum sativum* and *Nicotiana glutinosa* test plants produced no visible symptoms but they were subsequently found to be infected using ELISA. The symptomless infection in *N. benthamiana* test plants in this study could be due to several factors. Temperature, daylength, humidity and other factors known to influence symptom development in inoculated plants could not be controlled in the large open plant barn used.

CONCLUSION

Research into African cassava mosaic disease (ACMD) in Africa has been limited almost entirely to cassava and only little attention has been given to the possible role of other plants (both cultivated and wild) as alternative hosts of the virus that causes the disease. Thus, there is limited information on natural hosts of ACMV in Ghana and elsewhere in sub-Saharan Africa as a whole. The present study has shown that ACMV can spread, via the *Bemisia tabaci* vector, from infected cassava to ten local accessions of *J. curcas*, thus establishing it as a host of the virus in Ghana. It is, therefore, possible for ACMV to be transmitted from *J. curcas* to healthy cassava plantings. It may, therefore, be necessary to screen *J. curcas*, both cultivated and wild, for ACMV and other Begomoviruses that infect cassava and other crops.

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