Effect of a cowpea mild mottle virus isolate on growth and yield of bambara groundnut *(Vigna subterranea* L.)

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Abstract

A virus isolated from naturally infected bambara groundnut was identified as an isolate of cowpea mild mottle virus on the basis of biological and physical properties, particle morphology and serology. The isolate infected 13 of 16 plant species from five families. Virus particles were flexous elongated rods measuring 630-650 × 13 nm. The virus affected the growth and yield of three varieties of bambara groundnut tested. Biomass production was decreased by about 50 per cent in both field and screen-house grown plants. The yield of all three varieties examined were also reduced (20%) by the virus, although the varieties showed different degrees of susceptibility.

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Introduction

Cowpea mild mottle (CMMV) carlavirus has been isolated from a number of leguminous crops including cowpea *(Vigna unguiculata* L.), soybean *(Glycine max* L.), winged bean *(Psophocarpus tetragonolubus* L.), groundnut *(Arachis hypogea* L.) and mung bean *(Vigna radiata)* (Brunt & Kenten, 1973; Fauquet *et al.*, 1979; Thouvenel *et al.*, 1982; Lizuka *et al.*, 1984; Anno-Nyako, 1984). It has also been isolated from tomato (Brunt & Philips, 1981).

CMMV has a very extensive geographical distribution and a wide host range. It occurs in Africa, Asia, South America and Oceania (Jeyanandarajah & Brunt, 1993). Differences in host ranges of isolates from different locations and crops have been reported and, in some cases, these isolates have exhibited different degrees of effect on crop species. Two strains of CMMV were recently described and characterized in India. Although both strains affected groundnut, they did so to different extent and were consequently referred to as severe and mild mottle strains of CMMV (Sivaprasad *et al.*, 1990; Sivaprasad & Sreenvivasula, 1996).

Other studies conducted in India showed that different strains of CMMV could decrease pod yield of groundnut by 53-86 per cent. Although CMMV was first isolated from cowpea and described in Ghana (Brunt & Kenten, 1973), there has been no study conducted to examine the importance of this virus on leguminous crops grown in Ghana. This paper describes a CMMV isolate from bambara groundnut and its effect on the crop.

Materials and methods

Isolation of virus

The virus was first isolated from bambara groundnut plants grown in an experimental plot at the Crop Science Department, University of Ghana, and subjected to several single lesion transfers in *Chenopodium amaranticolor* Coste Reyn. Inocula prepared from single lesions were used to establish infection in bambara groundnut plants. Plants were grown in 30 cm diameter plastic pots containing heat sterilized sandy loam soils. The plants were grown under screen house conditions with temperature range of 27-31 °C.

Host range

Inoculum was prepared by grinding leaves from infected bambara groundnut plants in 0.067 *M* Sorensen's phosphate buffer with a *p*H 7.5

(tissue:buffer ratio, 1:10) with celite (3 mg ml^{-1}) incorporated as an abrasive. The plants tested included 16 species from nine genera and five families (Table I). At least five plants of each species were inoculated and replicated at least three times. Both inoculated leaves and uninoculated leaves of each species were assayed for virus recovery 7-14 days after inoculation by sap transmission to *C. amaranticolor.*

Electron microscopy

Virus samples were mounted on formvar/ carbon-coated grids and examined with a JEOL, JEM-100SX transmission electron microscope after staining with aqueous ammonium molybdate (AM) *p*H 7.0. Immunosorbent electron microscopy (ISEM) and decoration tests were made as described by Hill (1984) and Milne & Luisoni (1987) with some modifications. Antisera to cowpea mild mottle virus, cowpea aphid-borne mosaic virus and soybean mosaic virus were supplied by the Danish Government Institute of Seed Pathology, Denmark. Extracts of the bambara groundnut leaves infected with CMMV were reacted with the three antisera and examined under the EM. Similarly, extracts of leaf tissues with known infection of cowpea mild mottle virus, cowpea aphid borne potyvirus or soybean mosaic potyvirus were prepared and mounted on grids precoated with homologous antisera, decorated and also examined under the EM.

Effect of virus on growth and yield of bambara groundnut

Two experiments (field and pot) were conducted to examine the effect of CMMV on the growth and yield of three varieties of bambara groundnut. Seeds were sown in the field initially at three per hill and later thinned to one, and arranged according to a randomised block design with four replications and six treatments (three cultivars either CMMV infected or initially CMMV-free). There were six rows of five plants. Spacings were 20 cm within rows and 50 cm between rows, 60 cm between plots and 1 m between blocks. Peripheral bambara groundnut plants in each plot were used as guard rows. Insecticides (Actellic 25 EC; Zeneca Agrochemicals) were applied routinely to prevent white fly infestation. A parallel experiment was set up in the screen house. Seeds were sown in 25 cm × 30 cm plastic pots containing heat sterilized sandy loam soil and arranged in a randomised complete block on benches in the screen house. Inoculum was prepared from CMMV infected leaves of bambara groundnut and mechanically applied onto adaxial surface of designated plants in each replication. Plants were inoculated 2 weeks after germination. Control plants were sham-inoculated with distilled water.

Data were collected from three plants in each of the four inner rows. Height (ground level to tip of petiole) of plants and sizes of leaves were measured every week, following inoculation of plants for a period of 12 weeks, and the leaf area estimated using the formular:

$ALA = 0.71ELA + 0.23$ (Nguy-Ntamag, 1995)

where ALA is actual leaf area and ELA estimated leaf area. Number of pods were recorded at maturity (70 days after sowing) and dry shoot and seed weight measured. The extent of shriveling in the seed lots were also estimated.

Results and discussion

Symptoms and host range

The virus infected 13 species in three families which included both legumes and non-leguminous species (Table 1). Necrotic local lesions were produced on inoculated leaves of *C. quinoa* and *C. amaranticolor* within 5 days of inoculation. Unlike the isolate characterized by Brunt & Kenten (1973), the bambara groundnut isolate induced more distinct and prominent symptoms on C. *amaranticolor* than on C. *Quinoa* indicating that it could be a different isolate from the cowpea isolate (Brunt & Kenten, 1973) and the groundnut isolates characterized in India (Sivaprasad & Sreenivasulu, 1996). Plants systemically infected showed mosaic or mottling within 7 -14 days after inoculation.

TABLE 1

Host Range and Symptomatology of Cowpea Mild Mottle Virus isolated from Bambara Groundnut

		Symptom[1D]	
Family	*Species*	*Local*	*Systemic*
Chenopodiaceae	*C. amaranticolor*	N	-
	C. quinoa	N	-
Compositae	*Lactuca sativa*	-	-
Cruciferae	*Brassica oleracecae*		
	var. *capitata*	-	-
Leguminosae	*Glycine max*		
	cv. Henon 145	-	M, C, Ld
	Davis		M, C, Ld
	Tax 536-05		M, C, Ld
	Phaseolus lunatus		
	cv. Henderson bush white	-	M
	Henderson bush black	-	M
	Henderson bush brown	-	-*
	P. vulgaris		
	cv. Pinto	-	C, R
	Top crop	-	C, R
	Vigna radiata	-	C, Ld
	V. mungo	-	C
	V. unguiculata		
	Accra market-2	-	Mn, Ld
	Asontem	-	-*
	IT 83 S-818	-	Mm
	V. subterranea	-	
	CS-88-05		M, Ld
	Ex Ada	-	M, Ld
	Zimbabwe brown	-	M, Ld
Solanaceae	*Capsicum annum*	-	-
	Lycopericon esculentum	-	R
	Nicotiana benthamiana	-	M
	N. glutinosa	-	M
	N. tabacum	-	-

C = chlorosis
R = rugose symptoms
Ld = leaf distortion
- = no symptoms and virus was not recovered
-* = no symptoms but virus was recovered
Mm = mild mottle
M = mosaic
N = Necrotic lesions

Electron microscopy

Flexous elongated rods measuring 630-650 nm were observed under the electron microscope. Most of the rods were fragmented. The particles reacted positively with antiserum to cowpea mild mottle virus but not with the other antisera used in the ISEM test which confirmed their identity as a carlavirus.

Effect of virus infection on growth and yield of bamabara groundnut

The CMMV isolate affected the growth of all the three varieties of bambara groundnut examined. Growth reduction was observed in the plants in the 6th week and was consistent in all

the virus infected plants throughout the subsequent growing period (Fig. 1). Although leaf area index of field grown plants increased throughout the 12 week period, there was an appreciable reduction in leaf area in the inoculated plants (Fig. 2). Leaf area index of the virus-infected plants measured about 50 per cent compared to that of the uninfected controls, indicating a

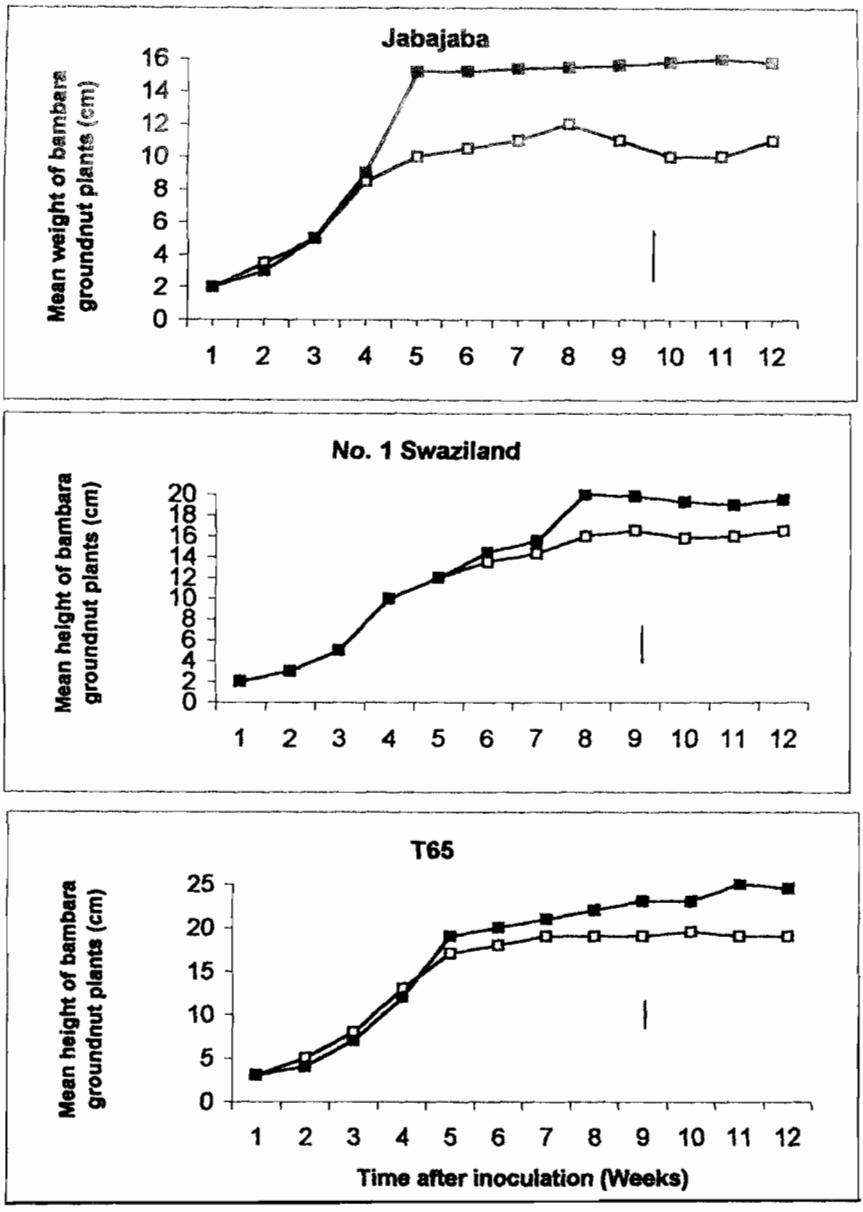

Fig. 1. Height of bambara groundnut plants either inoculated with CMMV ☐ or uninoculated ■ (Bar represents $P < 0.05$).

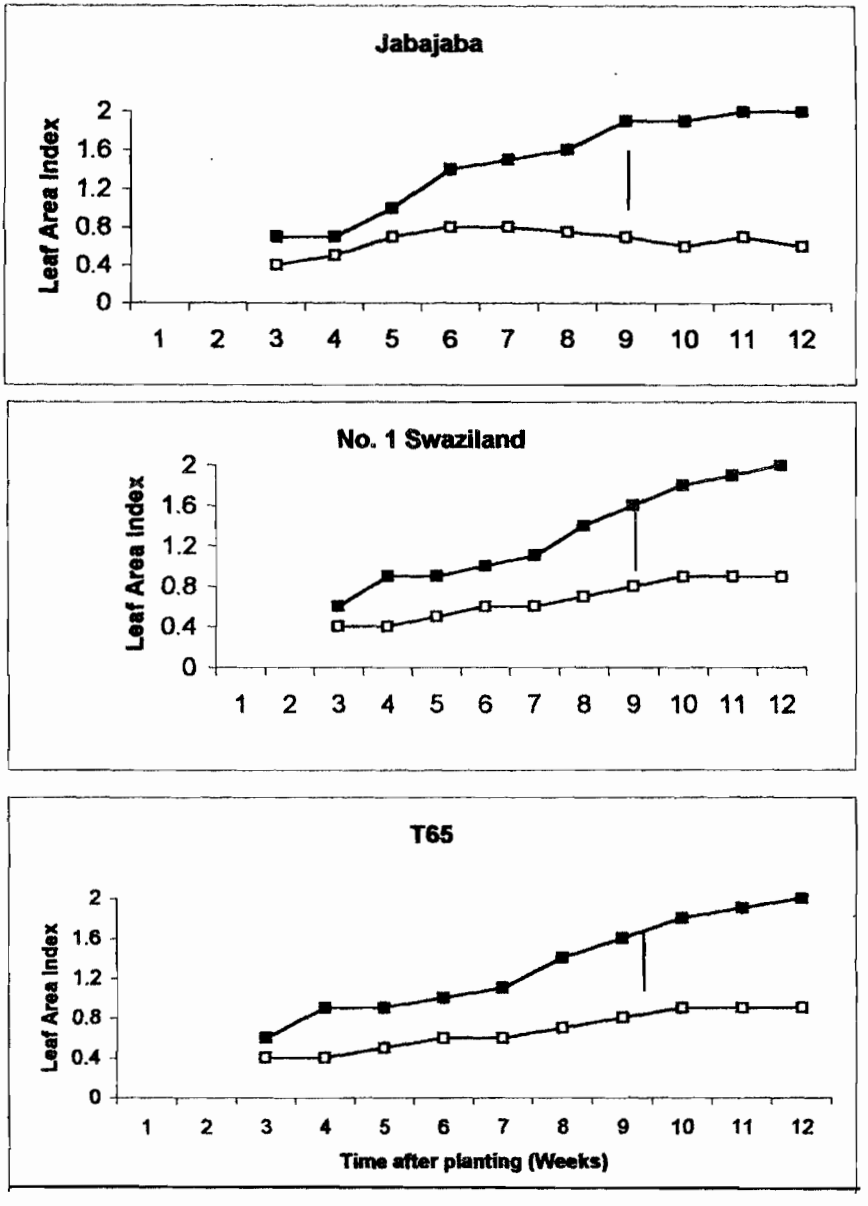

Fig. 2. Leaf area index of bambara groundnut plants either inoculated with CMMV isolate ☐ or uninoculated ■ (Bar represents $P < 0.05$).

marked effect of the virus on the crop. The leaf area decline observed in the inoculated Jabajaba plants could be due to the pronounced leaf drop in this variety as a result of the CMMV infection.

The leaf area index of CMMV-infected plants were reduced by about 70 per cent for the three varieties by the end of the experimental period. Leaf area reduction due to viral infections of

TABLE 2

Shoot Dry Weight and Mean Pod Production of Inoculated (+virus) or Uninoculated (-virus) Bambara Groundnut Plants

Variety	*Shoot dry weight*			*Mean number of pods*		
	+Virus	*-VIrus*	*LSD*	*+Virus*	*-Virus*	*LSD*
			(P < 0.05)			*(P < 0.05)*
Field experiment						
Jabajaba	12.23 ± 0.27	25.67 ± 1.36	7.6	8.13 ± 0.57	28.54 ± 0.87	9.7
Swaziland 1	7.26 ± 0.73	12.56 ± 0.98	3.7	11.13 ± 0.61	21.42 ± 0.52	5.6
T65	8.61 ± 0.39	17.8 ± 0.67	5.0	8.00 ± 0.76	21.50 ± 0.53	6.1
Pot experiment						
Jabajaba	6.37 ± 0.46	13.65 ± 0.73	5.9	4.22 ± 0.38	10.80 ± 1.93	3.7
Swaziland I	5.61 ± 0.76	9.11 ± 0.67	3.7	6.20 ± 0.61	13.50 ± 1.20	2.6
T65	7.10 ± 0.43	12.66 ± 0.86	4.8	5.30 ± 0.75	9.65 ± 0.90	3.1

TABLE 3

Shoot Grain Yield of Inoculated (+virus) or Uninoculated (-virus) Bambara Groundnut Plants

Variety	*Mean seed weight*			*100 seed weight*		
	+Virus	*-Virus*	*LSD*	*+Virus*	*-Virus*	*LSD*
			(P < 0.05)			*(P < 0.05)*
Field experiment						
Jabajaba	54.95 ± 2.43	70.53 ± 1.87	5.1	26.88 ± 0.97	31.45 ± 1.23	4.2
Swaziland 1	48.95 ± 1.67	56.86 ± 2.89	7.3	26.88 ± 0.91	38.30 ± 1.30	6.2
T65	51.26 ± 2.73	68.57 ± 1.59	9.7	28.83 ± 0.73	32.99 ± 1.56	5.2
Pot experiment						
Jabajaba	34.67 ± 2.10	50.87 ± 2.98	9.7	16.53 ± 0.87	27.76 ± 1.37	6.3
Swaziland I	25.63 ± 1.89	39.53 ± 2.53	5.3	17.61 ±0.78	29.10 ± 2.75	7.3
T65	23.45 ± 1.23	36.71 ± 2.2	6.3	13.51 ± 0.68	23.13 ± 0.93	7.8

leguminous plants have been reported by other workers (Shoyinka *et al.*, 1978, 1979; Bailiss & Senanayake, 1985; Thottapilly & Rossel, 1992). Drabo *et al.* (1995) observed that the biomass of bambara groundnut was decreased by 63 per cent due to infection by viruses in Burkina Faso.

In the current study, pod and seed yield were also reduced in the virus-infected plants (Tables 2 and 3), but the extent of decreased yield varied with variety. Yields from CMMV-infected Jabajaba plants were consistently lower than yields from the other two varieties. It appears there may be some varietal differences in susceptibility, and this is being investigated. A higher proportion of seeds harvested from CMMV-infected plants (12% 18%, 34%, respectively, for T65, No.1 Swaziland and Jabajaba) were wrinkled compared to seeds from the uninfected control plants (9%, 8%, 34%). This might have contributed in part to the decrease in seed weight. The mottling and mosaic symptoms developed on the leaves might have reduced the photosynthetic area and, hence, the available assimilate for partitioning into the developing seed. This may have contributed to

the decrease in weight of the seeds. Further experiments are, however, required to confirm and expand these points. The fact that seed transmission of CMMV has been reported in some leguminous crops (Iwaki *et al.*, 1982; Fauqet & Thouvenel,1987) transmitted by *Bemisia tabaci* (Muniyappa & Reddy, 1983; Costa *et al.*, 1983) and widely distributed makes CMMV an important virus that can affect legume production.

REFERENCES

Anno-Nyako, F. O. (1984) *Identification and partial characterisation of a mild mottle disease in soybean* (Glycine max *L.*) *in Nigeria.* (PhD Thesis.) University of Science and Technology, Kumasi, Ghana.

Bailiss, K. W. & Senanayake, S. (1985) Reproductive losses induced by virus infection in determinate flowering faba bean *(Vicia faba* L.). *Acta Phytopath. Scientiarum hung.* **20**, 47-58.

Brunt, A. A. & Kenten, R. H. (1973) Cowpea mild mottle, a newly recognised virus infecting cowpeas (*Vigna unguiculata* L.) in Ghana. *Ann. appl. Bioi.* **74**, 67-74.

Brunt, A. A. & Philips, S. (1981). 'Fuzzy vein' a disease of tomato (*Lycopersicon esculuntum*) in Western Nigeria induced by cowpea mild mottle virus. *Trop. Agric., Trin.* **58**, 177-180.

Costa, A. S., Gasper, J. O. & Vega, (1983) Mosaico angular to feijoeiro jalo causado por urn "carlavirus" transmitido pela mosca branca Bemesia tabacina. *Fitopathol. Bras.* **8,** 325-327.

Drabo, I., Sereme, P. & Dabire, C. (1995) Country Report. In *Proceedings of Workshop on Conservation and Improvement of Bambara Groundnut* (Vigna subterranea *(L.) Verdc.) 14-16 November 1995 Harare, Zimbabwe.* pp. 19-26.

Fauquet, C., Lamy, D. & Thouvennel, J. C. (1979) Viral diseases of wing bean in Ivory Coast. *FAO Pl. Prot. Bull.* **27**, 81-87.

Fauquet, C. & Thouvennel, J. C. (1987) *Plant Viral Diseases in the Ivory Coast.* Paris: ORSTOM. Institut Francais de Recherche Scientifique pour le Developpement en Cooperation, Collection Initiation--Documentations Techniques No. **46**.

Hill, S. A. (1984) Methods in Plant Virology. In *Blackwell Scientific Publications* (T. F. Preece, ed.). London: 164 pp.

Iwaki, M., Thongmeearkon, P., Prommin, M., Honda, Y. & Hibi, T. (1982) Whitefly transmission and some properties of cowpea mild mottle on soybean in Thailand. *Pl. Dis.* **66**, 365-368.

Jeyanandarajah, P. & Brunt, A. A. (1993) The natural occurrence, transmission, properties and possible affinities of cowpea mild mottle virus. *J. Phytopath.* **137**, 148-156.

Lizuka, N., Rjeshwari, R. J., Reddy, D. V. R., Goto, F., Munyappa, V., Bharathan, N. & Ghanaker, A. M. (1984) Natural occurrence of a strain of cowpea mild mottle virus on groundnut *(Arachis hypogea)* in India. *Phytopath.* Z. **109**, 245-253.

Milne, R. G. & Luisoni, E. (1977) Rapid immune electron microscopy of virus preparations. *Meth. Virol.* **6**, 265-281.

Muniyappa, V. & Reddy, D. V. R. (1983) Transmission of cowpea mild mottle virus by *Bemesia tabacina* in a non-persistent manner. *Pl. Dis.* **67**, 391-393.

Nguy-Ntamag, F. E. (1995) Country Report. In *Proceedings of Workshop on Consevation and Improvement of Bambara Groundnut* (Vigna subterranea *(L.) Verde.) 14-16 November 1995 Harare, Zimbabwe.* pp. 27- 29.

Shoyinka, S. A., Bozarth, R. F., Reese, J. & Okunsanya, B. O. (1979) Field occurrence and identification of southern bean mosaic virus (cowpea strain) in Nigeria. *Turrialba* **29**, 111-116.

Shoyinka, S. A, Bozarth, R. F., Reese, J. & Rossel, H. W. (1978) Cowpea mottle virus a seedborne virus with distinctive properties infecting cowpeas in Nigeria. *Phytopath.* **68**, 693-699.

Sivaprasad, V. & Sreenivasulu, P. (1996) Characterisation of two strains of cowpea mild mottle virus naturally infecting groundnut *(Arachis hypogaea* L.) in India. *J. Phytopath.* **144,** 19-23.

Sivaprasad, V. & Saigopal, D. Y. R, Sreenivasulu, P. & Nayudu, M. Y. (1996) Seed transmission of a carlavirus naturally infecting groundnut *(Arachis hypogea* L.) in India. *J. Pl. Dis. Prot.* **97**, 584-550.

Thottappilly, G. & Rossel, H. W. (1992). Viruses diseases of cowpea in tropical Africa. *Trop. Pest Mgmt* **38**(4), 337-348.

Thouvenel, J. C., Monstsarrat, A. & Fauquet, C. (1982) Isolation of cowpea mild mottle virus from diseased soybeans in Ivory Coast. *Pl. Dis.* **66**, 336-337.