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Towards the control of a severe form of cassava mosaic disease in Nigeria: diagnostic survey for cassava mosaic begomoviruses

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Abstract. A diagnostic survey was conducted in the southern states of Nigeria to determine if the Ugandan strain of *East African cassava mosaic virus* (EACMV-UG2) has spread to Nigeria. Farmers' fields (290) were visited in which 946 cassava leaf samples were collected in addition to 220 samples of whitefly vectors. The overall impression of cassava mosaic disease (CMD) symptom severity was recorded as mild, moderately severe or severe. Polymerase chain reaction (PCR) tests were conducted on the samples using nucleotide primers for the detection of *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and EACMV-Ug2. CMD symptoms in most farms in Abia, Cross River, Ebonyi, Ekiti and Imo States were either moderately severe or severe. About the same number of farms had moderately severe and severe symptoms in Ogun, Ondo, Osun and Oyo States. CMD symptoms were mild in most farms in Akwa Ibom, Anambra, Delta, Edo, Enugu and River States. ACMV is the dominant species, occurring singly in 62.2% of samples while EACMV alone was detected in 1.1%. Mixed infections by the two viruses occurred in 22.4% of samples and the remaining 14.3% tested negative to the primers. The two viruses were also detected in the whitefly vectors. Most of the plants doubly infected with ACMV and EACMV were characterised by severe symptoms. A higher proportion (49%) of farms with mixed infections were observed in the survey area compared to the 14.3% recorded in a previous survey conducted in 1998. Biological variants of ACMV were observed of which some induced very severe symptoms. EACMV-UG2 specific primers tested negative to all the samples. Although EACMV-UG2 may not occur in the area surveyed, virulent strains of ACMV and EACMV seemed to occur. The extremely severe symptoms induced by some isolates of ACMV and by the two viruses in mixed infections underscore the need to make available to farmers, desirable resistant cassava genotypes to sustain cassava production in Nigeria.

Introduction

Cassava mosaic disease (CMD) is prevalent in sub-Saharan Africa (Cours-Darne, 1968; Hahn *et al*., 1980; Fargette *et al*., 1988; Otim-Nape *et al*., 1998) and it is the major disease of cassava in Nigeria. In Africa, the disease is caused by *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) (Bock and Woods, 1983; Hong *et al*., 1993) and *South African cassava mosaic virus* (SACMV) (Berrie *et al*., 1998). The viruses belong to the family *Geminiviridae* genus *Begomovirus* (Mayo and Pringle, 1998). ACMV was the only known virus in Nigeria until 1999 when EACMV was reported (Ogbe *et al*., 1999). Yield losses due to CMD can be as high as 90% on susceptible cassava genotypes (Terry and Hahn, 1980).

An outbreak of a severe form of CMD occurred in Uganda in the 1990s, which led to very low cassava yields in that country. The severe form of the disease was caused by a virulent strain of EACMV known as the Ugandan variant (EACMV-UG2) (Zhou *et al*., 1997; Pita *et al*., 2001a). Extremely severe symptoms are induced when EACMV-UG2 and ACMV co-infect cassava (Harrison *et al*., 1997). Recent reports confirmed the presence of EACMV-Ug2 in the Democratic Republic of Congo (DRC) and Congo Republic and (Neuenschwander *et al*., 2002), which indicate its movement towards West African countries where ACMV predominates. The occurrence of EACMV, however, had been reported in Cameroon, Nigeria, Togo, Ghana, Ivory Coast and Guinea (Fondong *et al*., 1998; Winter, 1998; Ogbe *et al*., 1999; Offei *et al*., 1999; Pita *et al*., 2001b). In a bid to protect cassava production in Nigeria, IITA initiated a project on pre-emptive management of the severe form of CMD due to virulent strains of the viruses. An aspect of this project is to provide a situation report on CMD and the various cassava mosaic begomoviruses associated with the disease through a diagnostic survey.

Materials and Methods

A diagnostic survey was conducted in 16 southern states out of the 36 states in the country between December 2002 and August 2003. The States are: Abia, Akwa Ibom, Anambra, Bayelsa, Cross River, Delta, Ebonyi, Edo, Ekiti, Enugu, Imo, Ogun, Ondo, Osun, Oyo and River. The survey routes were pre-determined using the road map of Nigeria and the routes were selected to cover as much area of each state as possible. Cassava leaf and whitefly-vector samples were collected at an interval of between 10 and 15 km. In each farm visited, the overall impression of symptom severity of CMD was recorded as mild, moderately severe or severe. 290 farmers' fields were visited in which 946 cassava leaf samples were collected. In addition, 220 samples of whitefly vectors were collected. Extraction of DNA from the leaf samples was conducted as reported by Dellaporta *et al*. (1983) and polymerase chain reaction (PCR) tests were conducted (Zhou *et al*., 1997) using the following nucleotide primers for the detection of ACMV, EACMV and EACMV-UG2:

ACMV primers: ACMV-AL1/F (GCG GAA TCC CTA ACA TTA TC) and ACMV-AR0/R (GCT CGT ATG TAT CCT CTA AGG CCT G) (Zhou *et al*., 1997); EACMV primers: UV-AL3/F (TAC ACA TGC CTC RAA TCC TG) and UV-AL1/R2 (CTC CGC CAC AAA CTT ACG TT) (Zhou *et al*., 1997); EACMV-UG2 primers: UV-AL1/F1 (TGT CTT CTG GGA CTT GTG TG) and ACMV-CP/R3 (TGC CTC CTG ATG ATT ATA TGT C) (Harrison *et al*., 1997). EACMV-UG2 DNA was used as positive control. Dr James Legg of IITA, Uganda supplied the DNA sample under permission of the Nigerian Plant Quarantine, Moore Plantation, Ibadan, Nigeria.

Results and Discussion

CMD symptoms were either moderately severe or severe in most farms in Abia, Cross River, Ebonyi, Ekiti and Imo States (Table 1). The number of farms with either moderately severe or severe symptoms was about the same as the number of farms with mild symptoms in Ogun, Ondo, Osun, Oyo and River States. CMD symptoms were mild in most farms in Akwa Ibom, Anambra, Delta, Edo and Enugu States. Only three farms were visited in Bayelsa State and CMD in them was mild

ACMV was the dominant virus species, occurring singly in 62.2% of the 946 samples tested while EACMV was detected in 1.1%. Mixed infections by the two viruses occurred in 22.4% of samples and the remaining 13.9% of the samples tested negative to the primers. Ogbe (2001) reported a similar trend and mixed infections of the two viruses have also been reported (Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001a). Most of the samples that tested negative to the three primers used were collected from asymptomatic plants while a few samples were

from plants showing symptoms. Between 20% and 42% of plants sampled in Anambra, Cross River, Ebonyi, Enugu, Imo, Ondo and Osun States had mixed infections (Table 2) while other states recorded less than 20% of plants doubly infected by the two viruses. The lowest proportion of plants with mixed infections occurred in Edo State (9.8%). The

Table 1: The status of cassava mosaic disease severity in farmers' fields in the southern states of Nigeria as determined by a diagnostic survey for cassava mosaic begomoviruses.

State	No. of farms	CMD severity		
		Mild (%)	Moderately severe (%)	Severe (%)
Abia	17	13.5	35.3	41.2
Akwa Ibom	11	63.6	36.4	0.0
Anambra	9	88.9	11.1	0.0
Bayelsa	3	100	0.0	0.0
Cross River	21	28.6	57.1	14.3
Delta	23	82.6	17.4	0.0
Ebonyi	13	30.8	61.5	7.6
Edo	31	71.0	22.6	6.4
Ekiti	16	37.5	25.0	37.5
Enugu	14	78.6	7.1	14.3
Imo	23	26.1	52.2	21.7
Ogun	31	54.8	22.6	22.6
Ondo	17	47.1	35.3	17.6
Osun	23	56.5	21.7	21.7
Oyo	30	50.0	43.3	6.7
River	8	50.0	37.5	12.5

Table 2: The proportion of cassava plants in farmers' fields infected by *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) as determined by a diagnostic survey in Southern States of Nigeria.

State	Plants infected by ACMV (%)	Plants infected byACMV+EACMV (%)
Abia	75.0	16.7
Akwa Ibom	72.7	15.1
Anambra	64.0	28.0
Bayelsa	87.5	0.0
Cross River	64.7	25.0
Delta	70.0	14.3
Ebonyi	63.4	19.5
Edo	71.9	9.8
Ekiti	61.2	17.9
Enugu	47.8	40.9
Imo	49.4	42.3
Ogun	56.9	17.4
Ondo	43.7	39.1
Osun	54.9	32.4
Oyo	70.5	11.6
River	79.2	12.5

cassava plants mixed infected by ACMV and EACMV are widespread in the survey area (Figure 1). This therefore provides suitable environment for screening cassava genotypes for resistance to CMD. Most of the plants doubly infected by the two viruses were characterised by severe symptoms (Figure 2) as previously reported (Ogbe, 2001; Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001a). This might partly account for the number of farms showing either moderately severe or severe symptoms in Cross River, Ebonyi, Imo, Ondo and Osun States. Representative samples demonstrating mixed infections are presented in Figure 3. The six cassava leaf samples in lanes 1–6 and 11–16 (Figure 3a) tested positive to both viruses. The EACMV primers also detected the DNA of EACMV-UG2 that was used as positive control. However, the EACMV-Ug2 was differentiated from the Nigerian isolates of EACMV by the EACMV-UG2 specific primers (Figure 3b). This implies that the virulent Ugandan variant (EACMV-UG2) might not be present in the part of Nigerian surveyed. This corroborates earlier report by Ogbe (2001).

ACMV was also the most frequently detected virus in the samples from

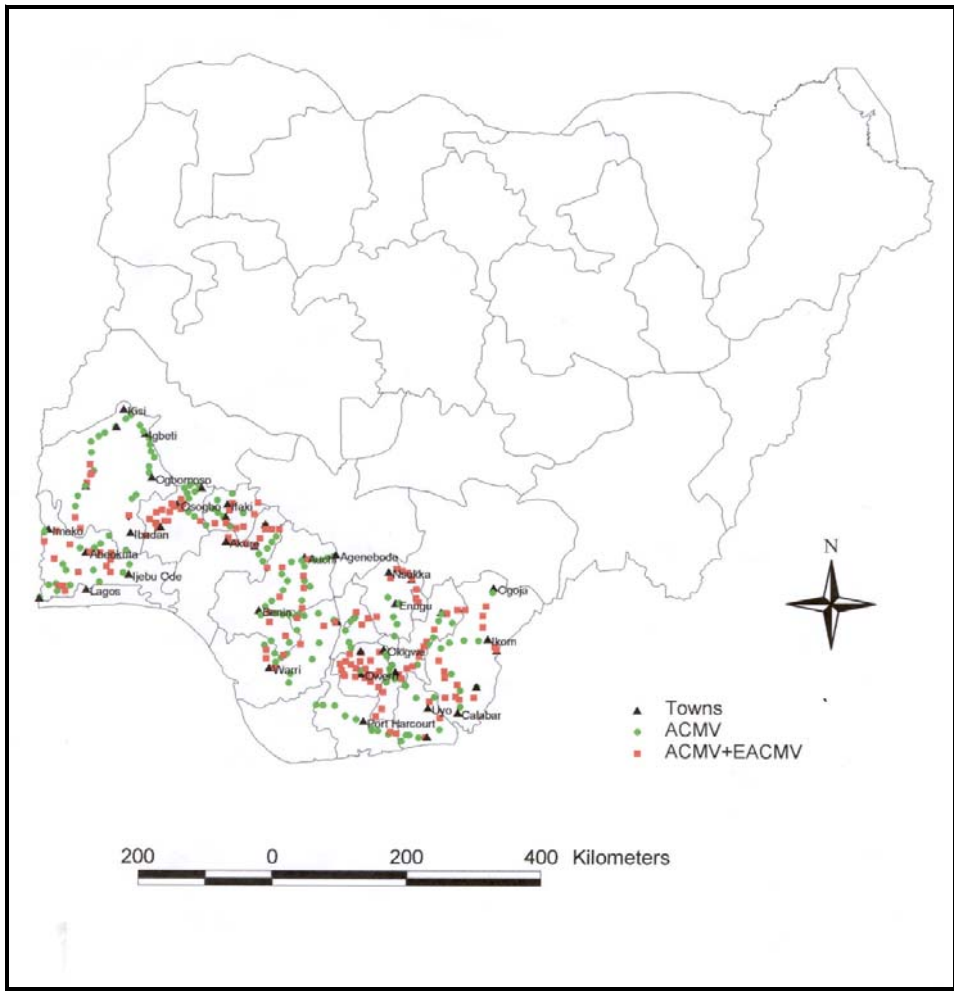

Figure 1: The distribution of African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) in farmers' fields in Southern Nigeria.

a b c

Figure 2: Cassava plants (a, b) showing severe symptoms of cassava mosaic disease due to mixed infections by *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) and a plant (c) that is symptom-free.

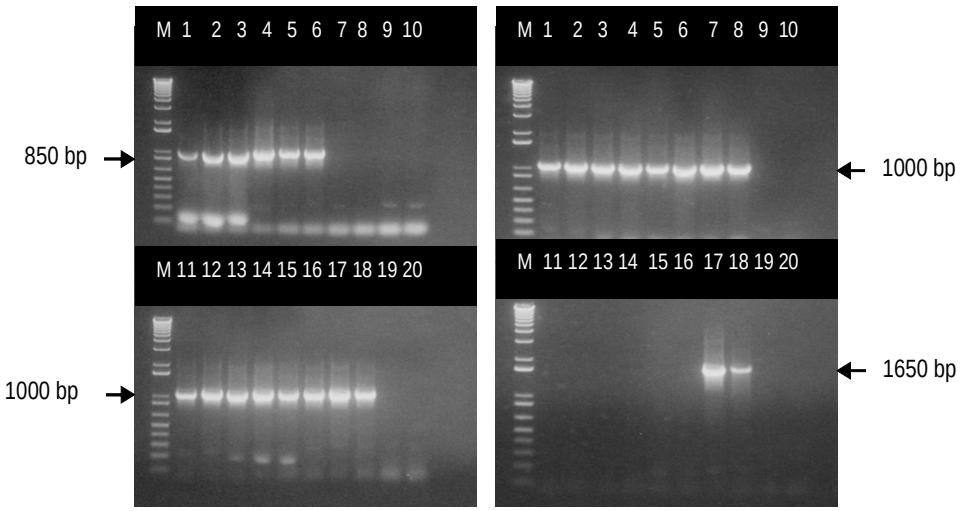

Figure 3: African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) DNA fragments amplified by polymerase chain reaction and analysed by gel electrophoresis[1]

[1] The viruses doubly infected cassava plants in farmers' fields in the Southern States of Nigeria.

M=1kb plus marker; lanes 1–6 contained the DNA of cassava leaf samples 75, 242, 503, 507,647, 796, respectively; lanes 7 and 8 contained the DNA of virulent Ugandan strain of *East African cassava mosaic virus* (EACMV-Ug2) as positive control; lane 9 contained DNA of healthy cassava leaf as negative control; lane 10 contained DNA extraction buffer as negative control. This arrangement was repeated for lanes 11–16; 17 and 18; 19, 20, respectively.

Figure 3a, lanes 1–10 was tested by ACMV primers ACMV-AL1/F/AR0/R; lanes 11–20 were tested by EACMV primers UV-AL3/F/AL1/R2. Figure 3b, lanes 1–10 were tested by EACMV primers UV-AL3/F/AL1/R2; lanes 11–20 were tested by EACMV-Ug2 primers UV-AL1/F1/ACMV-CP/R3.

asymptomatic plants followed by mixed infections of ACMV and EACMV (Table 3). Only two asymptomatic samples contained EACMV alone. Most asymptomatic samples in Cross River, Delta, Edo, Ekiti, Ogun, Osun and Oyo States were virus-free suggesting that most visually healthy plants in those states were actually healthy. In contrast, most samples from asymptomatic plants in Abia, Ebonyi, Enugu and Imo States were infected with either ACMV or EACMV. This could be a reflection of the CMD pressure, which might be higher in these states than in Cross River, Delta, Edo, Ekiti, Ogun, Osun and Oyo. It could also imply that more resistant cassava genotypes are cultivated in Cross River, Delta, Edo, Ekiti, Ogun and Oyo. It was observed during the survey that most cassava fields in Cross River, Delta, Edo, Ekiti, Oyo, Ogun and Osun States contained moderately resistant (TMS 30572, TMS 30555) and resistant (TME 1) genotypes. The detection of ACMV and EACMV in asymptomatic plants (Table 3) is important information in the determination of CMD incidence. It implies that CMD incidence is often underestimated.

A higher proportion (49%) of farms with mixed infections were observed in the survey area as against the 14.3% recorded in a previous survey conducted in 1998 (Ogbe, 2001). More farms and more plants per farm were sampled in the 2003 survey than in the 1998 survey in order to increase the chances of detecting EACMV-UG2, which was the object of the survey. This might partly accounted for the higher number of farms with mixed infections in 2003 than in 1998. Intensive farming and the dissemination of the viruses by whitefly-vectors could also bring about the higher frequency of farms with mixed infections. The two viruses were detected in the whitefly-vectors and at a high frequency in Enugu and Imo States where the highest number (40-42%) of plants with mixed infections was observed.

Two biological variants of ACMV were distinguishable based on symptoms induced on the same cassava genotype in the same

Table 3: Detection of *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) in asymptomatic cassava plants in the Southern States of Nigeria.

State	No. of plants	Number of samples testing positive to:			
		ACMV	EACMV	ACMV +EACMV	No. of virus-free plants[1]
Abia	13	9	–	1	3 (23.1)
Akwa Ibom	7	3	–	–	4 (57.1)
Anambra	5	2	–	1	2 (40.0)
Bayelsa	1	–	–	–	1 (100)
Cross River	9	2	–	–	7 (77.8)
Delta	15	6	–	–	9 (60.0)
Ebonyi	10	7	–	–	3 (30.0)
Edo	15	2	–	–	13 (86.7)
Ekiti	11	1	–	–	10 (90.9)
Enugu	12	4	–	5	3 (25.0)
Imo	13	8	–	1	4 (30.8)
Ogun	24	2	–	–	22 (91.7)
Ondo	4	2	1	–	1 (25.0)
Osun	11	2	1	–	8 (72.7)
Oyo	18	3	–	–	15 (83.3)
River	4	2	–	–	2 (50.0)

[1] Numbers in parenthesis are percentages.

field. One variant induced a yellow and green mosaic pattern with mild leaf distortion while another variant induced a combination of a bleaching effect and green coloration with notable leaf distortion (Figure 4). Both variants were widely distributed and in some instances occurred in mixed infections leading to severe symptoms. Ogbe *et al*. (2003) had earlier reported the occurrence of biological variants of ACMV on a test plant *Nicotiana benthamiana* Domin and also serological variants of the virus. There are indications of the occurrence of strains/variants of cassava begomoviruses in the survey area. There were some ACMV isolates that reacted differently to the different ACMV primers tested (Table 4). There were also some isolates that could not be identified by primers that detect ACMV, EACMV, *Indian cassava mosaic virus* (ICMV) and *South African cassava mosaic virus* (SACMV). The isolates also did not react with a pair of universal primers that could detect whitefly-transmitted geminiviruses of cassava, cowpea, okra and tomato. These isolates need to be characterised further and they could be novel strains of cassava mosaic begomoviruses. Genetic modification of species of geminiviruses has produced new

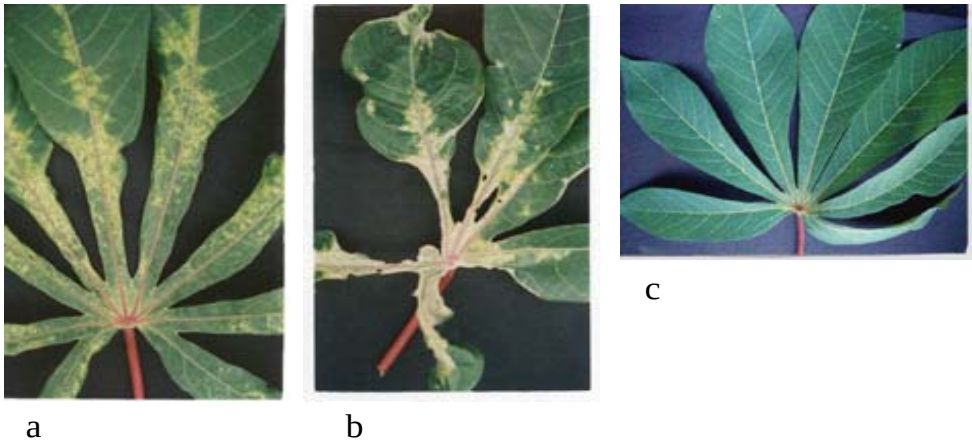

Figure 4: Characteristic symptoms of biological variants *of African cassava mosaic virus* on cassava leaf: yellow and green mosaic pattern with mild leaf distortion (a), combination of bleaching effect and green coloration with notable leaf distortion (b), symptom-free leaf (c).

Table 4: Representative reactions of variants of cassava begomoviruses in cassava leaves collected from farmers' fields in the Southern States of Nigeria.

Primer	Target virus	Infected cassava leaf sample number			
		389 (3)	422 (4)	605 (3)	706 (3)
ACMV-AL1/AR0/R	African cassava mosaic virus (ACMV)	+	–	–	–
JSP001/002	ACMV	+	–	+	–
UV-AL3/F/AL1/R2	East African cassava mosaic virus	–	–	–	–
ICMV/F/R	Indian cassava mosaic virus	–	–	–	–
SACMVCP3/CP	South African cassava mosaic virus	–	–	–	–
UNIVERSAL A/B[1]	Whitefly-transmitted geminivirus	+	+	+	–

[1] The primers detect whitefly-transmitted geminiviruses of cassava, cowpea, okra and tomato.
The numbers in parentheses are the symptom severity scores based on a 5-point scale (1 being no symptoms and 5 being severe mosaic and distortion of entire leaf (Terry, 1975).

species and strains such as EACMV-UG2 (Zhou *et al*., 1997) and SACMV (Berrie *et al*., 1998).

Conclusion

Between 43% and 76% of farms visited in 10 of the 16 states had CMD ratings of either moderately severe or severe. This is an indication that in the southern states of Nigeria where most of the crop is cultivated an epidemic of CMD could occur if resistant genotypes are not widely cultivated. It is, however, worthy of note that the Nigerian farmers practise the cultivation of mixtures of resistant and susceptible genotypes to forestall the entire farm being devastated by CMD. This possibly explains why an epidemic of CMD has not occurred despite the high proportion of mixed infections and the occurrence of virulent variants of cassava mosaic begomovirus in the survey area. The challenge, therefore, is to reduce the number of susceptible cassava genotypes that are being cultivated by the farmers. The project on the pre-emptive management of a severe form of CMD is, therefore, timely to provide additional resistant cassava genotypes to the Nigerian farmers to forestall any outbreak of a virulent recombinant and to also minimise the impact of EACMV-Ug if it eventually spread to Nigeria.

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