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Full Length Research Paper

Rate of re-infection of tissue culture-derived Latin American and East and Southern African cassava genotypes by mosaic disease

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The rate of reinfection by cassava mosaic disease (CMD) in initially virus-free cassava plants of two Latin American and twelve East and Southern African cassava genotypes grown was studied under high disease pressure conditions. An improved clone, TMS 4(2)1425, from the International Institute of Tropical Agriculture was used as check. The virus-free plants had been produced through meristem-tip culture and multiplied in a pest-proof screen house. The genotypes were planted in single row plots of 5 plants each, arranged in a randomized complete block design with 4 replications and spacing of 1 × 1 m^2. Incidence and severity of CMD on the genotypes were assessed weekly, from 4 to 16 weeks after planting (WAP). Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) tests for the cassava mosaic virus were carried out using young leaves collected randomly at 15, 16 and 17 WAP from plants both with and without symptoms. Six genotypes had > 60% CMD incidence at 4 WAP; by 7 WAP, 12 genotypes had > 60% incidence. Only *Kigoma red*, *Kiroba*, and UKG-41-6 were not infected at 4 WAP while *Mbudumali* had 90% incidence at this time. At 16 WAP, ten genotypes had 100% CMD incidence; *Kigoma Red* was 39.6% infected. ELISA detected a mean CMD reinfection rate of 66.6%; PCR detected 69%. A high negative and significant (*P*< 0.01) correlation (r = - 0.70) was established between CMD severity and storage root yield.

Key words: Virus-free cassava genotypes, tissue culture, rate of reinfection, cassava mosaic disease.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), an important root and tuber crop in the tropics (Pujol et al., 2002; Meireles da Silva et al., 2003) is affected by diseases and pests that constitute a major constraint to achieving its full production potential in Africa. Losses in tuber yield due to diseases can be as high as 90% (Wydra and Msikita, 1998). Cassava mosaic disease (CMD) is the most important cause of yield loss in Africa. CMD resistant varieties either do not get infected, or when they do, they sustain little or no damage. Such varieties have been widely used to control CMD. Resistant varieties may not always be available to farmers or may not have all the other preferred attributes. This is why susceptible varieties are still widely grown, especially in low CMD-pressure areas where adoption of resistant varieties to sustain production is not a must. A basic approach to CMD control is the use uninfected propagules for new plantings. Healthy stem cuttings establish more readily and grow faster than infected ones. The subsequent yields of initially healthy plants are also substantially greater, even if when they get infected by whiteflies during growth (Fargette et al., 1988; Thresh et al., 1994a). Moreover, the use of healthy cuttings together with crop hygiene means that initially there are no foci of infection within or alongside new plantings from which

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Abbreviations: CMD, Cassava mosaic disease; **WAP,** weeks after planting; **ELISA,** enzyme-linked immunosorbent assay; **PCR,** polymerase chain reaction; **IC-PCR,** immunocapture-polymerase chain reaction; **GLM,** general linear model; **ACMV,** African cassava mosaic virus.

Table 1. Tissue culture-derived cassava genotypes used in the trial.

Genotype	Origin
IAC-12	Brazil, Latin America
IAC-14	Brazil, Latin America
Simonye	Southern Africa
Nachinyaya	Tanzania, East Africa
UKG-41-6	Tanzania, East Africa
Kibaha	Tanzania, East Africa
Kiroba	Tanzania, East Africa
Mbundumali	Malawi, Southern Africa
Silira	Malawi, Southern Africa
Bangweulu	Zambia, Southern Africa
Mkondezi	Malawi, Southern Africa
L9/304/47	Zambia, Southern Africa
Kigoma Red	Tanzania, East Africa
Maunjili	Malawi, Southern Africa
TMS4(2)1425	IITA, Ibadan, Nigeria

spread can occur. This avoids, or at least delays, the onset of CMD and decreases the period over which spread can occur during the early and most vulnerable stages of the plant growth.

Even though virus-free plants of susceptible genotypes can be produced through meristem-tip culture (Kartha and Gamborg, 1975; Ng et al., 1992), they get reinfected (Thro et al., 1998) at a high rate under conditions of high inoculum pressure; under specific low-disease pressure, reinfection may not occur or the rate may be low (Akano et al., 1997). *In vitro* clean up of susceptible varieties may therefore, be a valuable short-term emergency measure to reduce infection and can be effective in low disease-pressure areas. Information on varietal differences for ability to remain clean after virus therapy followed by exposure to open field conditions is scanty in cassava (and most crops). Against this background, this study was designed to study the response of some virus-free Latin American and Southern Africa cassava genotypes (previously introduced to Nigeria) to CMD. The objective was to determine the rates of CMD reinfection on the genotypes under high inoculum pressure and to assess the relationship between the disease and yield.

MATERIALS AND METHODS

Planting materials

Twelve genotypes from East and Southern Africa, two from Latin America, and one improved cultivar from IITA (check) were used in the trial (Table 1). Stem cuttings were obtained from virus-free cassava plants produced through meristem-tip culture and used to establish a field experiment in a randomized complete block design with four replications. The stem cuttings were planted on ridges spaced at 1m apart; interplant spacing along the ridges was also 1 m. Each genotype was planted in a plot consisting of five plants.

Assessment of incidence and severity of CMD

Incidence and severity of CMD were recorded at weekly intervals from 4 to 16 weeks after planting (WAP). Incidence was recorded as the proportion of plants showing symptoms over the total number of plants in a plot expressed as percentage. Severity of CMD was scored on a scale of 1 to 5 (IITA, 1990) where 1 = no symptoms and 5 = severe mosaic distortion, twisted and misshapen leaves on ≥ 80% of the leaflets.

Virus detection

Virus detection was done using triple antibody sandwich enzyme-linked immunosorbent assay (Thomas et al., 1986) and immunocapture-polymerase chain reaction (IC-PCR). Young leaf samples were collected from three randomly selected plants per plot (including diseased and symptomless) from 15 to 17 WAP. The values were read 24 h after incubation from the Dynex MRX ELISA plate reader at 4°C and were used to quantify the virus. A sample was considered infected when the mean absorbance value (virus concentration) of the wells containing the sample was ≥ 1.5 times that of the healthy sample. The check for this experiment included healthy and virus infected plant samples. At 12 months after planting, the trial was harvested and storage root yield determined.

Data analysis

The data on incidence and severity of CMD and storage root yield were subjected to analysis of variance using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 2003). Correlation analysis was run between storage root yield at 12 MAP and CMD severity score at different WAPs. Also, the correlation between storage root yield at 12 MAP and mean CMD severity score was determined.

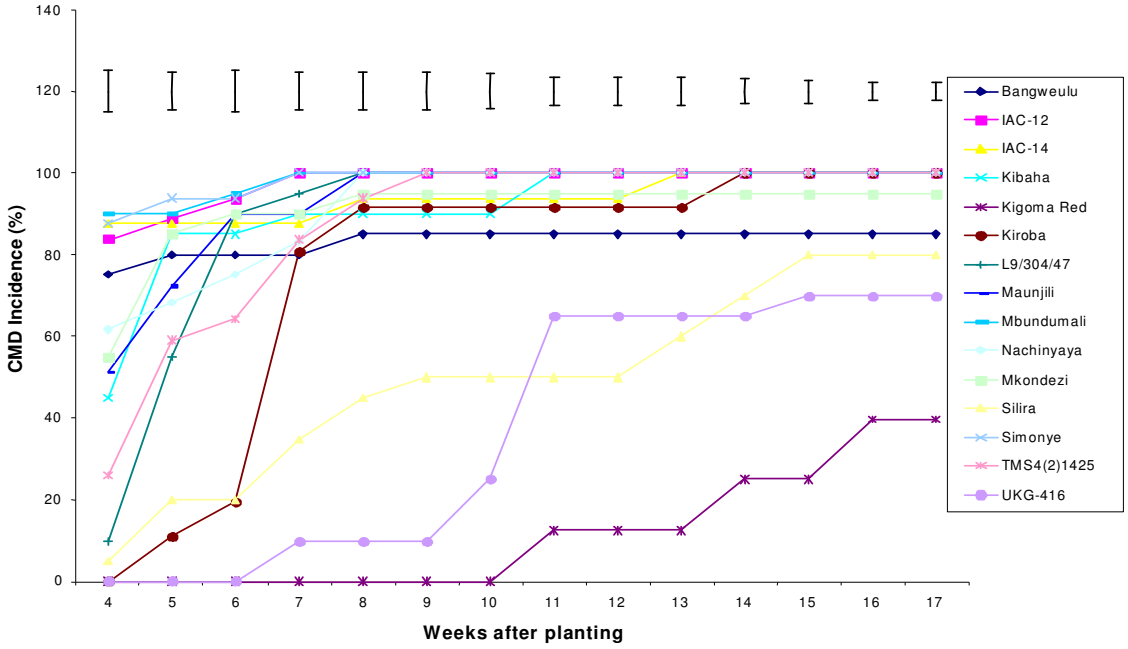

Figure 1. Progress curve of cassava mosaic disease incidence on 15 genotypes of cassava between 4 to 16 weeks after planting.

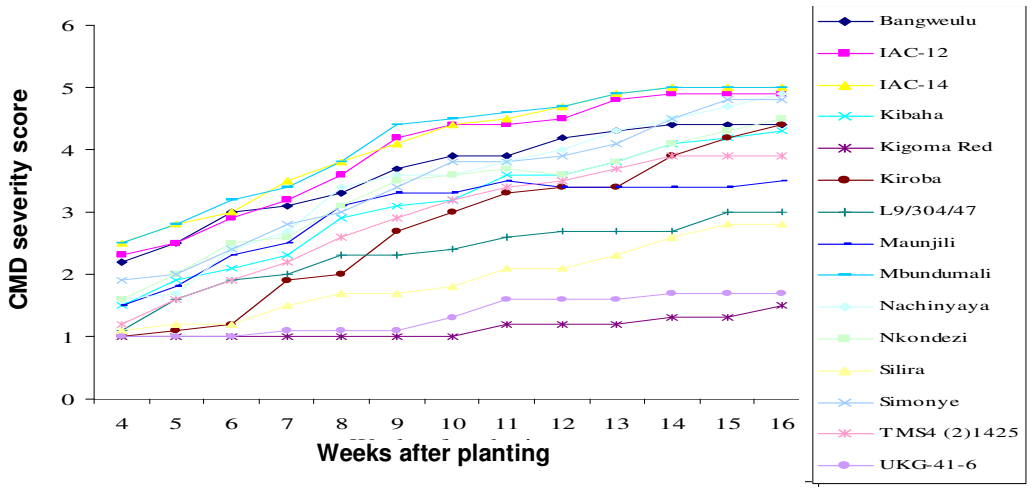

Figure 2. Progress curve of cassava mosaic disease severity on 15 genotypes of cassava between 4 to 16 weeks after planting.

RESULTS

CMD incidence and severity

Six out of the 15 genotypes had a high disease incidence of more than 60% at 4 WAP; 12 genotypes showed incidence of above 60% at 7 WAP. CMD incidence at 4 WAP ranged from 0% in *Kigoma Red*, *Kiroba*, and *UKG-41*-6 to 87.5% in IAC-14 and *Simonye*, and 90% in *Mbundumali*. Symptoms were first observed in *Kigoma Red* at 11 WAP. At 16 WAP, 10 genotypes had attained the maximum incidence (100%); by this time *Kigoma Red* had only 39.6% incidence (Figure 1). There were significant differences ($P < 0.01$) among genotypes for disease severity at all sampling dates (Table 1). At 4 WAP, *Kiroba*, UKG-41-6, and *Kigoma Red* did not have CMD symptoms; the most severe symptoms by this time (score 2.5) were on IAC-14 and *Mbundumal* (Figure 2). By 16 WAP, *Kigoma Red* had mild symptoms (score 1.5), while *Mbudumali* (score 5.0), IAC-14 (score 5.0),

Table 2. Mean storage root yield (12 months after planting), mean cassava mosaic disease (CMD) severity of 15 cassava genotypes established with initially disease-free cuttings and correlation between yield and CMD severity at different weeks after planting (WAP).

Genotype	**IAC-12**	**IAC-14**	***Simonye***	***Nachinyaya***	**UKG-41-6**	***Kibaha***	***Kiroba***	***Mbundumali***
CMD severity	4.0	4.1	3.5	3.5	1.3	3.1	2.7	4.1
Yield (t/ha)	7.5	6.0	19.2	16.3	30.5	20.7	17.8	4.5
Genotype	**Silira**	**Bangweulu**	**Mkondezi**	**L9/304/47**	**Kigoma Red**	**Maunjili**	**TMS4 (2)1425 (check)**	
CMD severity	1.9	3.6	3.3	2.3	1.1	3.0	2.9	
Yield (t/ha)	31.8	14.2	22.9	34.2	20.4	25.3	16.6	

Correlation coefficient values between yield and CMD severity											
4WAP	**5WAP**	**6WAP**	**7WAP**	**8WAP**	**9WAP**	**10WAP**	**11WAP**	**12WAP**	**13WAP**	**14WAP**	**15WAP**	**16WAP**
- 0.806 ***	- 0.743 ***	- 0.699 ***	- 0.753 ***	- 0.599 **	- 0.750 ***	- 0.760 ***	- 0.748 ***	- 0.767 ***	- 0.770 ***	- 0.755 ***	- 0.704 ***	- 0.702 ***

CMD severity scored on a 1 - 5 scale (IITA, 1990).

Nachinyaya (score 4.9), and IAC 12 (score 4.9) had the most severe symptoms. Progress in severity of the disease in UKG-41-6 was similar to that in *Kigoma Red* (Table 2).

Relationship between yield and CMD

Highly significant ($P < 0.001$) negative correlation was observed between storage root yield and CMD severity at all sampling dates (Table 2). The highest correlation ($r = -0.81$) was observed at 4 WAP. The three genotypes with mean disease severity scores of ≥ 4.0 (Table 2) had the lowest yields; IAC-12 (7.5 t/ha), IAC-14 (6.0 t/ha), and *Mbundumali (*4.5 t/ha). Nevertheless, the *Kigoma Red*, that had the lowest mean disease severity score did not have the highest yield; L9/304/47 (disease score of 2.3) had the highest yield (34.2 t/ha). Genotypes that attained maximum disease incidence early also tended to have lower yields (especially when the incidence was coupled with high severity) than those that attained it later. However, high incidence tended to have less impact on storage root yield if the severity was low as in *Silira*, UKG-416, and *Kigoma Red*.

Detection of African cassava mosaic virus (ACMV) using ELISA and PCR

ELISA did not detect ACMV in UKG-41-6 and *Kigoma Red* from 15 to 17 WAP (Table 3) but detected it in *Mbundumal*i, *Simonye*, *Kibaha*, TMS 4(2)1425, *Nachinyaya*, *Mkondezi*, IAC-12, IAC-14 and *Bangweulu*. L9/304/47 and *Silira* tested negative at 15 and 17 WAP. However, PCR analysis detected the presence of ACMV in *Kiroba* at 15 WAP and *Kigoma Red* at 16 WAP, and confirmed absence of the virus in L9/304/47 and *Silira* at 15 and 17 WAP. ELISA detected a CMD reinfection rate of 66.6% while PCR detected69%.

DISCUSSION

The significant differences observed among genotypes for CMD severity at all sampling dates suggest that the different varieties responded differently to CMD infection. According to Thresh and Cooter (2005), cassava varieties differ greatly in their response to CMD; some are severely stunted and produce little or no yield of foliage, stem cuttings or tuberous roots, whereas others are relatively unaffected and sustain little or no damage.

Genotypes from Latin America had very high reinfection rates and this had a serious impact on their storage root yields. This was expected because CMD does not occur in Latin America and therefore germplasm from that region has not been improved for CMD resistance, the exposure to the new disease shocked them more than it did the African adapted germplasm. Cours (1951) reported a and storage root yield. This study also showed a tendency for varieties infected early to have lower yields than those that were infected later. This is expected because the earlier the infection, the longer the plant-disease interaction that translates into greater negative impact on yield. Similar observations were made by Fargette et al. (1988) who reported that plants grown from CMD-infected cuttings were more severely

Table 3. Absorbance values read from ELISA plate and status of cassava mosaic virus detected in young leaf samples from 15 to 17 weeks after planting (WAP).

Genotype	Absorbance value			Virus characterization*		
	15 WAP	16 WAP	17 WAP	15 WAP	16 WAP	17 WAP
Bangweulu	0.22	0.50	0.51	+	+	+
IAC-12	0.25	0.40	0.45	+	+	+
IAC-14	0.29	0.52	0.63	+	+	+
L9/304/47	0.11	0.25	0.14	-	+	-
Maunjili	0.25	0.38	0.29	+	+	+
Mbundumali	0.28	0.36	0.54	+	+	+
Nachinyaya	0.21	0.25	0.41	+	+	+
Mkondezi	0.28	0.27	0.47	+	+	+
Silira	0.14	0.19	0.12	-	+	-
UKG-41-6	0.14	0.10	0.11	-	-	-
Kibaha	0.24	0.29	0.19	+	+	+
Kigoma Red	0.10	0.09	0.11	-	-	-
Kiroba	0.13	0.31	0.32	-	+	+
Simonye	0.23	0.36	0.47	+	+	+
TMS 4(2)1425	0.28	0.40	0.28	+	+	+
Positive control	0.36	0.56	0.63			
Negative control	0.10	0.10	0.11			
LSD (0.05)	0.13	0.26	0.22			

*+ Presence of the virus indicated mean absorbance value equal to or greater than 1.5 times the absorbance of the healthy sample (negative control)- Absence, indicated by the mean absorbance value lower than 1.5 times the absorbance of the healthy sample (positive control).

affected than those of the same variety infected at an early stage of crop growth by whitefly; plants infected late sustained little or no damage. Although there was a negative relationship between CMD severity and storage root yield, the varieties (*Kigoma Red* and UKG-41-6) that had the lowest mean CMD scores with low incidence did not have the highest yield. This suggests that yield was a function of both genotype and CMD incidence/ severity with possible genotype by CMD interaction effect, further confirming the observations made by Thresh and Cooter (2005). Despite the fact that the variety L9/304/47 was infected early and recorded more than 70% incidence, it had the highest yield. This may partially explain why farmers have continued to grow tolerant varieties (like this one) because they can still give good storage root yield despite the disease. Farmers would only consider a variety susceptible (and discard it) to a disease if the interaction between the latter two impacts negatively on yield. There is a need to investigate the role of such tolerant varieties as a source of inoculum in the out-break break of CMD epidemics.

The severe symptoms expressed by genotypes from Latin America and East and Southern Africa may be related to virus concentration (Thresh et al., 1994b; Fargette et al., 1996). This was observed in *Mbundumali*, IAC-14, and *Bangweulu* with a high virus concentration and severe disease symptoms. Resistant varieties when infected show mild symptoms that are restricted to some shoots (Jennings, 1960; Fargette et al., 1996) and recover with age (Njock et al., 1996) with reduced symptoms and a low virus concentration (Fargette et al., 1996) as in *Kigoma Red* and UKG-41-6. It is possible that the varieties which tested negative for the virus during these periods of evaluation have the ability to recover from infection. However, a non-consistent result was obtained from other genotypes in which the virus was not detected at 15 and 16 WAP but tested positive at 17 WAP.

The rates of reinfection are high under high disease pressure (Akano et al., 1997) as observed in this study with a high CMD reinfection rate of 87.5% at 4 WAP. Pozzer et al. (1994) observed a high reinfection rate of 80% in healthy sweet potato. There was, however, a rapid increase in severity in most genotypes though obtained from virus-free cassava plants produced through meristem-tip culture. This is an indication that such genotypes are highly susceptible. Reinfection therefore occurs if the genotypes are susceptible and inocula are present.A CMD reinfection rate of 66.6% was observed when determined using ELISA, while PCR results detected reinfection rate of 69%. This could be as a result of a reduction in symptoms over time and the ability of some genotypes to recover from infection.

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