Etiology of African Cassava Mosaic Disease

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ABSTRACT

Bock, K. R., and Woods, R. D. 1983. Etiology of African cassava mosaic disease. Plant Disease 67: 994-995.

Isolates of both the type and coast strains of cassava latent virus (CLV) were inoculated mechanically to the mosaic-sensitive cassava cultivar N Mex 55, in which they induced symptoms typical of African cassava mosaic disease. CLV was recovered from these infected plants by standard sap-transmission inoculations to *Nicotiana benthamiana* and gemini particles were subsequently detected in leaf-dip preparations of them, indicating this virus is in fact the causal agent of mosaic disease. Transmission rates were low (4/20 and 10/50 for CLV-C and CLV-T, respectively) when partially purified virus preparations from infected *N. benthamiana* were used as inocula but were comparatively high (11/26) when partially purified preparations of CLV-C from mosaic-affected cassava were used. It is apparent that the name cassava latent virus is no longer valid and that the pathogen should be referred to as cassava mosaic virus.

Although cassava latent virus (CLV) is apparently associated with African cassava mosaic disease and has been isolated from mosaic-affected plants only (1,3,4,7), past attempts to reinfect cassava (*Manihot esculenta*) with isolated virus by mechanical inoculation (1,3,7) by grafting (5), using dodder (5), or by means of the whitefly vector *Bemisia tabaci* (4) have been unsuccessful. In this paper, we report infection of cassava by mechanical inoculation using CLV in cassava sap and in partially purified preparations. These infections resulted in symptoms indistinguishable from those of cassava mosaic disease.

MATERIALS AND METHODS

Test plants. Cuttings of the cassava cultivar N Mex 55, known to be highly sensitive to African cassava mosaic disease (2), and derived from plants growing in quarantine isolation at the Kenya Agricultural Research Institute (altitude 2,100 m), were propagated in steam-sterilized soil in a roofed, glass-sided room at about 25 C without supplementary lighting. They were totally shaded from direct sunlight throughout the preinoculation growth period. Inoculations were made 11–14 days after cuttings were planted, when the first-formed immature leaves were not more than 2–3 cm long.

Inoculated plants were transferred to a glasshouse maintained insect-free by liberal use of seven Vapona insecticidal resin strips (2,2 dichlorovinyl demethyl-phosphate), which were replaced at monthly intervals. Glasshouse temperatures were not controlled and ranged from about 18 C daily minimum to about 25 C daily maximum. Test plant leaves were dusted lightly with Carborundum powder (600-mesh), inoculated by finger, and immediately rinsed with tap water. Final records and observations were made after 120 days. A total of 90 uninoculated cassava plants were used as controls. Inoculated cassava plants that developed mosaic symptoms were indexed for CLV on *Nicotiana benthamiana*.

Sources of inoculum. Bock et al (4) identified two serologically distinct strains of CLV, the type strain from western districts of Kenya (CLV-T) that also occurs in west Africa, and the coast strain (CLV-C), which occurs in coastal areas of Kenya. Three sources of CLV-C in cassava, obtained from the field in coastal districts of Kenya, were used in sap transmission experiments (Table 1, experiments 1–3). These were CLV-C1 from mosaic-diseased "tree" cassava (presumed to be an interspecific hybrid between *Manihot glaziovii* and *M. esculenta*) and from *M. esculenta* 'Kibandameno' affected by a severe (CLV-C2) or mild mosaic (CLV-C3).

Indexing to *N. benthamiana* and *N. debneyi*, a diagnostic host for cassava brown steak virus (CBSV) (*unpublished*), together with subsequent electron microscopy, indicated that the tree cassava source (CLV-C1) contained both CLV and cassava brown streak virus. The two cassava sources, CLV-C2 and CLV-C3, apparently contained CLV-C only.

N. benthamiana leaves from CLV-C-infected plants derived from a glasshouse isolate (CLV-C4) were used as the inoculum source in experiments 4 and 5.

Young shoots of mosaic-diseased cassava (cultivar Aipin Valenca) cuttings grown under glasshouse conditions were used for partial purification and concentration of CLV-C5 from cassava (experiments 6–9). Inoculation from this source to *N. benthamiana*, together with subsequent electron microscopy, indicated the presence of CLV-C only.

An isolate of the type strain of CLV (CLV-T1) described by Bock et al (3) and of the coast strain (CLV-C4) were propagated in *N. benthamiana* in glasshouses with a temperature range of 18–25 C. Experiments 10–19 were carried out with partially purified preparations of these strains.

Preparation of inoculum. For all sap-transmission experiments, leaf tissue was ground in 0.06 M phosphate buffer, pH 7.5, and the crude extract rubbed onto test plant leaves.

For partial purification and concentration of virus (experiments 6–10 and 17–19), systemically infected cassava or *N. benthamiana* leaves were homogenized in 0.06 M phosphate buffer containing 0.01 M sodium diethyldithiocarbamate and 0.1% thioglycollic acid, pH 7.5, an equal volume of a mixture of *n*-butanol and chloroform (1:1) was added, then the slurry was stirred for 10 min at laboratory temperature and clarified by centrifugation at 4,000 rpm for 10 min. The clear pale yellow supernatant was centrifuged at 78,000 *g* for 120 min and the pellets resuspended in 0.01 M phosphate buffer, pH 7.5, to give an approximate ratio of 2 ml resuspending buffer: 100 g initial leaf weight. In experiments 11–16, the method of purification described by Sequeira and Harrison (6) was used, the pellets being resuspended in 0.005 M tris-HCl buffer containing 0.0025 M ethylene diamine tetraacetic acid, pH 8.0, to give a similar buffer/initial leaf ratio.

In all experiments involving partially purified virus, the resuspended pellets were used directly as inoculum. A small aliquot (usually 0.2 ml) of the inoculum was examined under the electron microscope before and after clarification by centrifugation at 10,000 *g* for 2 min and the number of CLV gemini particles per field recorded. With all inocula, most if not all detectable virus was removed by the brief clarifying centrifugation.

Electron microscopy. Preparations for electron microscopy were mixed with two

Accepted for publication 20 February 1983.

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Table 1. Attempts to infect cassava with cassava latent virus from various sources

Experiment no.	Isolate	Source	Preparation	No. infected/ no. inoculated	Days to first symptoms	Suspending buffer	Estimated no. gemini particles/EM field
1	CLV-C1	Cassava	Sap	5/12	20–32	PO4	···
2	CLV-C2	Cassava	Sap	0/14	···	PO4	···
3	CLV-C3	Cassava	Sap	0/11	···	PO4	···
Total				5/37			
4	CLV-C4	*N. ben.*[a]	Sap	0/7	···	PO4	···
5	CLV-C4	*N. ben.*	Sap	0/6	···	PO4	···
Total				0/13			
6	CLV-C5	Cassava	p.p.[b]	3/7	26–28	PO4	100
7	CLV-C5	Cassava	p.p.	4/5	35–39	PO4	100
8	CLV-C5	Cassava	p.p.	0/6	···	PO4	100
9	CLV-C5	Cassava	p.p.	4/8	32–36	PO4	100
Total				11/26			
10	CLV-C4	*N. ben.*	p.p.	2/7	51,83	PO4	50
11	CLV-C4	*N. ben.*	p.p.	1/6	61	Tris-HCl	100
12	CLV-C4	*N. ben.*	p.p.	1/7	81	Tris-HCl	50
Total				4/20			
13	CLV-T1	*N. ben.*	p.p.	0/6	···	Tris-HCl	100
14	CLV-T1	*N. ben.*	p.p.	1/8	80	Tris-HCl	50
15	CLV-T1	*N. ben.*	p.p.	0/7	···	Tris-HCl	50
16	CLV-T1	*N. ben.*	p.p.	0/7	···	Tris-HCl	100
17	CLV-T1	*N. ben.*	p.p.	1/6	45	PO4	50
18	CLV-T1	*N. ben.*	p.p.	2/8	34, 39	PO4	100
19	CLV-T1	*N. ben.*	p.p.	6/8	34–53	PO4	100
Total				10/50			
Uninoculated experimental controls				0/90			

[a] *N. ben.* = *Nicotiana benthamiana.*
[b] p.p. = Partially purified preparation.

volumes of 2% sodium phosphotungstate, pH 6.8, and sprayed onto carbon-stabilized, necoloidine-coated, 200-mesh grids. Counts of the number of particles per field were made at ×40,000. All samples were examined in a Siemens 102 electron microscope.

RESULTS

No virus other than CLV was detected in any of the partially purified preparations. The results of the inoculations are summarized in Table 1. Partially purified preparations from both cassava (experiments 6–9) and *N. benthamiana* leaves (experiments 10–19) induced disease. None of the 90 control plants became infected.

Although symptoms were typical of African cassava mosaic disease in all infected plants, more plants were infected (11/26) and symptoms tended to appear significantly earlier and were more severe in plants inoculated with CLV obtained from the cassava source. Similar proportions of plants inoculated with isolated virus of the coast and type strains (4/20 and 10/50, respectively) were infected, though symptoms induced by the coast strain were generally more severe.

CLV was back-transmitted to *N. benthamiana* from all inoculated plants that developed mosaic symptoms and the presence of gemini particles was confirmed by electron microscopy of leaf-dip preparations.

DISCUSSION

The relatively high rate of transmission of the mosaic pathogen from cassava to cassava using standard sap-transmission techniques confirms the earlier results of Bock and Guthrie (2) and it seems unlikely that the presence of CBSV influenced transmission. The results also indicate that successful mechanical transmission is possibly dependent on source; leaf extracts from only one of three mosaic-affected cassavas resulted in transmission.

With partially purified preparations, there were differences in transmission rates between virus derived from affected cassava and virus derived from cultures in *N. benthamiana.* It was not possible to ascribe these differences or the differences in time required for first appearance of symptoms and subsequent severity to concentration of virus in inocula. Results indicate that there was no detectable difference in rate of transmission between inocula containing 100 particles per electron microscope field or those containing half that number. It appears that isolated CLV is transmitted to cassava only with difficulty.

It is likely that the success achieved in these experiments was attributable in part to the choice of cassava cultivar, preinoculation treatment of test plants, and careful inoculation procedures.

Although there is a recent report (6) of mosaic-affected plants in which CLV could not be detected by any of several techniques, our results indicate that cassava mosaic is caused by CLV. It is apparent that the name cassava latent virus is no longer appropriate and that in future publications, CLV should be correctly referred to as African cassava mosaic virus (ACMV).

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