Viruses Infecting Cassava in Kenya

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ABSTRACT

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A survey of cassava viruses was conducted in major cassava-growing regions of Kenya. A total of 185 leaf samples and 62 stem cuttings from plants with viral disease symptoms were collected and analyzed by biological, electron microscopy, enzyme-linked immunosorbent assay, and polymerase chain reaction. All samples from western Kenya had cassava begomoviruses (*African cassava mosaic virus* [ACMV], *East African cassava mosaic virus* [EACMV], and Uganda variant [EACMV-UG]) in either single or in mixed infection. However, all samples from the Coast region were infected with only EACMV, a begomovirus. In addition, 15 samples had mixed infections of EACMV and three other hitherto unidentified filamentous viruses. The viruses observed were 200, 500, 650, and 750 nm long, respectively. In addition to rod-shaped and some flexuous viruses, as seen in a crude sap preparation, pinwheels also were observed, indicating a possible association of some of the viruses with the *Potyviridae* family. The symptoms induced by these viruses in *Nicotiana benthamiana* were very severe and often caused about 50% death of the test plants. Back inoculation onto cassava resulted in 100% infections. This finding provides evidence that, other than begomoviruses that cause serious diseases of cassava in Africa, filamentous viruses also are present and, despite their limited distribution, they could reach local significance and, most probably, be as serious as begomoviruses. The implications of these findings are discussed and recommendations for future work suggested.

Additional keywords: carlavirus, CBSV, CMD, incidence, SDS-PAGE

Cassava (*Manihot esculenta* Crantz) is a key food crop in Coast and Western provinces of Kenya. Cassava was introduced to the East African coast in the eighteenth century by Portuguese sailors. The crop was not accepted at first but has now spread and gained prominence as a major food staple for many communities in sub-Saharan Africa (4). On a worldwide basis, cassava is ranked high among the top 10 most significant food crops produced in developing countries (9,10) and as a major source of carbohydrate for human consumption throughout the tropics, but particularly in Africa (4). Cassava is grown and consumed by the world's poorest and most food insecure households. In addition, it adapts to a wide range of uses: food security crop (consumed in fresh or processed form), cash crop, feed crop, and raw materials for industrial uses such as starch and alcohol production (13). Thus, cassava

constitutes an important source of income in rural and often marginal areas, and for women. The total world production in the year 2002 was 184,852,540 metric tonnes (t), with 100,689,149 t being produced in Africa alone (3). Cassava mosaic disease (CMD) has been a hindrance to cassava production in Africa throughout the twentieth century. In Kenya, CMD is caused by geminiviruses *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and Uganda variant (EACMV-UG) of the genus *Begomovirus*. The viruses are spread by the whitefly (*Bemisia tabaci* Gennadius) and by planting infected cuttings. Affected plants are stunted and have greatly diminished tuberous root yield. The disease has been a focus of extensive research in the past decade, to the extent that other cassava diseases were almost forgotten. Cassava brown streak disease (CBSD), caused by *Cassava brown streak virus* (CBSV), which probably belongs to the genus *Ipotomovirus* (*Potyviridae*), has been reported occurring on the East African coast (1). However, unlike CMD, the foliar symptoms of CBSD are less conspicuous and farmers often are unaware of the problem until the crop is harvested and the corky, yellow-brown necrosis in the roots becomes evident. CMD surveys have been conducted in Kenya (8) but none of them

took account of other cassava-infecting viruses in the area. Triggered by the CMD outbreak in the west of the country, we report here the findings of a survey of cassava-infecting viruses conducted in major cassava-growing regions of Kenya.

MATERIALS AND METHODS

Sample collection. During the period May to July 1999, a diagnostic survey for viruses infecting cassava was conducted in major cassava-growing regions of Kenya. The areas surveyed were the districts of Bungoma, Busia, Kakamega, Teso, and Vihiga in Western province; Suba and Siaya in Nyanza province; and Kilifi and Kwale in Coast province (Fig. 1). Cassava plants in farmers' fields were observed carefully for virus disease symptoms. To determine the types of viruses present in cassava, young cassava leaves as well as hardwood stem cuttings from plants with severe viral disease symptoms were collected.

For maintenance and propagation of viruses and virus isolates, the cuttings were planted in plastic pots in an insect-free glasshouse maintained at 23 to 27°C with a 16-h light period.

Inoculation of *Nicotiana benthamiana* and cassava. To determine the severity of symptoms induced by different viruses in *Nicotiana benthamiana*, plants at the three- to five-leaf stage were inoculated by sap extracted in 0.1 M phosphate buffer, pH 7.5, and by biolistic bombardment of total DNA (as described by Varrelman [15]) extracted from young virus-infected cassava leaves essentially as described by Dellaporta et al. (2). A batch of *N. benthamiana* plants inoculated with sap extracted from Kwale samples developed severe symptoms unusual for a begomovirus infection. CBSV was suspected as the putative pathogen and, consequently, a host range study was conducted for virus preliminary characterization. Sap and total DNA were extracted from cassava samples and inoculations were done on five plants of each of the following species: *Gomphrena globosa* L. (*Amaranthaceae*), *Chenopodium amaranticolor* Coste and Reynier, *C. quinoa* (*Chenopodiaceae*), *C. murale*, *Datura metel*, *D. stramonium* L., *Lycopersicon esculenta* (L.) karst., *N. benthamiana*, *N. clevelandii* Gray, *N. debneyi* L., *N. glutinosa*, *N. glutinosa* 24a, *N. tabacum* cv. Samsun NN, *Physalis floridana*

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Rydb., and *Solanum demisum* L. (Solana-ceae). Additionally, 20 and 6 *N. bentha-miana* plants were inoculated with nine virus isolates (Table 1) by sap and biolistic means, respectively. The plants were kept in the glasshouse for 2 to 3 weeks for symptom development.

CBSV propagation and purification. Virus propagation was done in *N. bentha-miana* and *N. glutinosa* 24a. The plants were harvested 3 to 4 weeks after inoculation with CBSV and leaves were frozen at -80°C until use.

Systemically infected leaves (200 g) of either plant were homogenized (1:3, wt/vol) in 0.5 M phosphate buffer, pH 7.5, and 0.5% Na_2SO_3 , then filtered through three layers of cheesecloth, after which 7% n-butanol was added, and the mixture was stirred for 1 h at 4°C and centrifuged at 10,000 rpm for 10 min at 4°C in a Sorvall GSA rotor.

Supernatant was adjusted slowly to 6% polyethyleneglycol (PEG) MW 6,000, and stirred for 1 h at 4°C . Precipitate was collected by low-speed centrifugation at 10,000 rpm for 10 min, and the pellet was resuspended in 0.02 M phosphate buffer, pH 8.2, containing 0.1% 2-mercaptoethanol and stirred overnight at 4°C . After centrifugation at 10,000 rpm, CsCl at 0.4 g/ml supernatant was added and thoroughly dissolved before being centrifuged at 45,000 rpm in a Beckman 70.1Ti rotor for 19 h. The opalescent bands were removed, diluted in 0.02 M phosphate buffer, pH 8.2, containing 0.1% 2-mercaptoethanol, and centrifuged at 55,000 rpm in a Beckman 70Ti rotor for 2 h. The final pellet was resuspended in 0.02 M Tris buffer, pH 8.2, and aliquots were taken for electron microscopy (EM) as well as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

SDS-PAGE. CBSV-infected leaf materials (from *G. globosa*, *N. benthamiana*, *N. clevelandii*, *N. debneyi*, *N. glutinosa*, and healthy *N. benthamiana*) were homogenized (1:10, w/vol) in protein extraction buffer (50 mM Tris, 5 mM boric acid, 5% glycerin, 1.5% polyclar AT, 0.01% 2-mercaptoethanol, pH 8.0) and centrifuged at

maximum speed for 30 s. The supernatant (about 10 μg of protein) and two virus preparations (P1 and P2) were subjected to 12% SDS-PAGE. The resolved proteins were stained in amido black (45% ethanol, 10% acetic acid, and 0.1% amido black 10B; cat. no. 2047; E. Merk AG, Darmstadt, Germany) for 20 min with gentle agitation. Excess stain was removed by immersing the gel for 1 h in several changes of destaining solution (25% isopropanol, 10% acetic acid, and 0.1% SDS).

EM. For adsorption preparations, leafy-samples were ground in buffer A (0.1 M Na/K-phosphate buffer, pH 7.0, containing 2% polyvinylpyrrolidone [wt/vol] and 0.2% Na_2SO_3 [wt/vol]) and drops of the resulting extract were placed on a piece of

parafilm. Pioloform-coated and carbon-stabilized nickel grids were floated on the leaf extracts or on drops of purified virus for 5 min, washed with 40 drops of distilled water, then stained with 5 drops of 1% uranyl acetate (UAc).

Immunosorbent electron microscopy. Immunosorbent electron microscopy (ISEM) was conducted essentially as described by Milne and Lesemann (6). Nickel grids were incubated for 5 min on drops of ACMV antiserum (or potyvirus antisera) diluted 1:1,000 (vol/vol) in buffer B (0.1 Na/K-phosphate buffer, pH 7.0, containing 0.05% NaN_3). The grids were washed with 20 drops of buffer C (0.1 M Na/K-phosphate buffer, pH 7.0) and floated onto leaf extracts or purified virus preparations

Fig. 1. Map of Kenya showing locations where cassava samples with viral disease symptoms were collected.

Table 1. Infectivity of nine cassava-infecting virus isolates to *Nicotiana benthamiana* by sap (I) and biolistic (II) inoculation

Sample no.	Sample origin (district)	Cassava ^a	Infected/inoculated		<i>N. benthamiana</i> ^b
			I	II	
2	Siaya	EACMV-UG	0/20	0/6	...
7	Kakamega	ACMV/EACMV	12/20	5/6	ACMV
65	Busia	ACMV	13/20	5/6	ACMV
112	Kilifi	EACMV	3/20	4/6	EACMV
119	Kwale	EACMV	^c 20/20	^d 2/6	EACMV/filamentous
123	Kwale	EACMV	^c 18/20	^d 3/6	EACMV/filamentous
124	Kwale	EACMV	^c 20/20	^d 2/5	EACMV/filamentous
125	Kwale	EACMV	^c 16/20	^d 2/6	EACMV/filamentous
126	Kwale	EACMV	^c 17/20	0/6	Filamentous

^a Virus detected in cassava. EACMV-UG, East African cassava mosaic virus, Uganda variant; ACMV, African cassava mosaic virus.

^b Virus detected in *N. benthamiana*.

^c Plants infected with a filamentous virus. EACMV could not be detected in these test plants.

^d Plants infected with only EACMV.

for 3 h at room temperature, washed with 40 drops of distilled water, then negatively stained with 5 drops of 1% UAc. All EM preparations were viewed in a Zeiss TEM 906 transmission electron microscope.

Enzyme-linked immunosorbent assay and polymerase chain reaction. Enzyme-linked immunosorbent assay (ELISA) was conducted essentially as described by Muniyappa et al. (7) using polyclonal antiserum raised against particles of ACMV for coating and monoclonal antibodies from our stock (DSMZ) that are specific for defining each of the begomoviruses (ACMV, EACMV, and EACMV-UG) for detection. Polymerase chain reaction (PCR) was conducted using primers described by Zhou et al. (16) while reverse transcription (RT)-PCR was conducted essentially as described by Langeveld et al. (5) for samples suspected to have CBSV.

RESULTS

Sample collection. In total, 185 leaf samples and 62 hardwood stem cuttings from farmers' fields (Fig. 1) were collected and analyzed. In western Kenya, a CMD incidence of up to 100% was observed in most farms while, at the coast, the incidence ranged from 25 to 50%.

PCR and ELISA. Every sample contained at least one cassava begomovirus (ACMV, EACMV, and EACMV-UG; Table 2). Additionally, results show that 52% of the samples from western Kenya were infected with EACMV-UG, 22% with ACMV, 17% with mixed infection (ACMV + EACMV-UG), 1.7% with mixed infection (ACMV + EACMV), and the rest with EACMV, which was the only begomovirus found in all coastal samples.

Host range and symptomatology. After sap extracts from cassava plants suspected of having a CBSV infection were inoculated to a series of test plants, several characteristic local and systemic symptoms were observed (Table 3). In *N. benthamiana*, symptoms of virus infection became visible 6 to 9 days after inoculation. They consisted of yellow net, vein banding, and systemic chlorosis (Fig. 2A) followed by yellowing of the entire plant, which sometimes led to the death of almost 50% of test plants (*data not shown*). When plants were inoculated at later stages (7- to 10-leaf stage), the number of lethal infections reduced significantly. *N. clelandii* produced yellow net, leaf deformation, systemic chlorosis, and plant death if inoculated at very early developmental stages, whereas *D. metel* showed vein banding followed by a slight leaf deformation; however, after the sixth week, the plant visibly recovered completely from the disease. *D. stramonium* produced chlorotic local lesions that turned necrotic as plants aged. *N. glutinosa* 24a produced vein banding, systemic chlorosis, leaf deformation, and stunting (Fig. 2C). Cassava produced green spots, vein banding,

chlorotic streaks on leaves and stem, motting, and a mosaic pattern (Fig. 2B and D). When sap extracted from cassava plants infected with a begomovirus in addition to the filamentous viruses was used to inoculate healthy *N. benthamiana* plants, only filamentous viruses (but not begomoviruses) were transmitted. However, biolistic inoculations using total DNA extracted from such plants resulted in only EACMV (but no filamentous virus) infections.

Description of particle morphology. Electron micrographs of crude sap extracted from any of the nine cassava plants (Table 1) revealed typical bisegmented icosahedral geminivirus particles (Fig. 3A). However, when crude sap of *N. benthamiana* previously inoculated with sap from five cassava plants (no. 119, 123, 124, 125, and 126) were examined, long, filamentous, flexuous rod-shaped particles resembling potyviruses or carlaviruses were observed (Fig. 4). A thorough EM investigation revealed, in addition to potyvirus-like particles (Fig. 4A), undefined rod-shaped particles (Fig. 4C and D) that were suspected to belong to either carlaviruses or potexviruses. Shorter particles observed were suspected of belonging to

other undescribed viruses or were probably fragments of broken virus or were possibly structures produced by host cells on infection by the viruses. The pinwheel structures (Fig. 4B), however, occasionally found in sap preparations strongly indicated that a member of the *Potyviridae* family was present in the cassava material. The length of the viruses could not be measured due to low virus concentration in crude sap. While in purified virus preparations, the integrity of the virions could no longer be guaranteed after purification. Nevertheless, based on the enlarged pictures of the few particles, particle length was estimated to be about 200 nm for the shortest, 500 nm for the medium flexuous, 650 nm for long and slightly flexuous, and about 750 nm for the longest flexuous rods. In partially purified virus preparations from *N. glutinosa* 24a, only carlavirus-like particles were observed (Fig. 3B), indicating that a third virus type might be present in the cassava plants.

Virus propagation and purification trials. Virus yields obtained by purifying either from *N. benthamiana* or *N. glutinosa* 24a were very low. Electron micrographs of virus preparations from *N. glutinosa*

Table 2. Viruses detected in cassava samples with varying levels of Cassava mosaic disease symptoms collected from different cassava-growing districts of Kenya

District	No. of samples	Viruses detected by ELISA, EM, or PCR ^a
Bungoma	10	EACMV-UG[KE]
Busia	55	ACMV, EACMV, and EACMV-UG[KE]
Kakamega	7	ACMV, EACMV, and EACMV-UG[KE]
Kilifi	19	EACMV and EACMVZ ^b
Kwale	32	EACMV and filamentous viruses
Siaya	28	EACMV-UG
Suba	11	ACMV, EACMV, and EACMV-UG
Teso	54	ACMV, EACMV, and EACMV-UG
Vihiga	31	ACMV and EACMV-UG

^a Enzyme-linked immunosorbent assay (ELISA); electron microscopy (EM); polymerase chain reaction (PCR); Kenyan isolate of *East African cassava mosaic virus*, Uganda variant (EACMV-UG[KE]); *African cassava mosaic virus* (ACMV).

^b *East African cassava mosaic Zanzibar virus*.

Table 3. Reaction of test plants upon inoculation with sap extracted from cassava infected with cassava filamentous viruses

Plant species	Symptom ^a	First symptoms visible (days)
<i>Chenopodium amaranticolor</i>	...	...
<i>C. murale</i>	...	...
<i>C. quinoa</i>	...	...
<i>Datura metel</i>	Ld, Vb	6
<i>D. stramonium</i>	Cs, LL, Ns	7
<i>Gomphrena globosa</i>	...	...
<i>Lycopersicon esculentum</i>	...	...
<i>Nicotiana benthamiana</i>	Pd, Sc, St, Py, Vb, Yn	6
<i>N. clelandii</i>	Ld, Pd, Sc, Ss, Yn	7
<i>N. debneyi</i>	Gs, Ld, LL, St	7
<i>N. glutinosa</i>	...	...
<i>N. glutinosa</i> 24a	Ld, Sc, St, Vb	10
<i>N. tabacum</i> Samsun NN	...	...
<i>Physalis floridana</i>	...	...
<i>Solanum demissum</i>	...	...
<i>Manihot esculenta</i>	Cs, CSt, G, M, Vb	9

^a Cs, chlorotic spot; CSt, chlorotic streaks; G, green spots; Ld, leaf deformation; LL, local lesion; M, leaf mosaic; Ns, necrotic spots; Pd, plant death; Sc, systemic chlorosis; St, stunting; Vb, vein banding; Yn, yellow net; ..., no symptoms.

revealed virus-like particles resembling carlaviruses (Fig. 3B), whereas those from *N. benthamiana* revealed particles resembling potyviruses (*data not shown*).

Determination of capsid protein sizes of putative cassava-filamentous viruses. SDS-PAGE of crude sap of virus-infected plants and purified virus preparations revealed two peptide bands of 38 and 34 kDa, in addition to the 54-kDa ribulose biphosphate carboxylase (RuBisCo) band present in the semipurified preparation (Fig. 5, lane 6) but absent in the second purification (Fig. 5, lane 7). It was not clear whether the 34-kDa peptide reflects an artefact which occurred during the purification process or resulted from partial proteolysis of the viral coat protein (CP). In some virus purifications (*data not shown*), an additional virus band of about 28 kDa was observed.

RT-PCR. The technique of RT-PCR was adopted as a possible method for specific detection of potyviruses. Sets of primers designed for the potyvirus group (5) were used for the RT-PCR tests; however, none of these primers amplified any products from the extracts of filamentous virus-infected plant tissues.

It was not possible to reach sufficient amounts of purified virus preparations

from either the carlavirus-like particles isolated from *N. glutinosa* 24a or other virus particles isolated from *N. benthamiana*; therefore, the identity of the virus or viruses remains an enigma. However, with the Koch's postulates verified, the virus or viruses should be considered true pathogens of cassava.

DISCUSSION

The results of the virus surveys in Kenya demonstrate a marked increase in prevalence, incidence, and severity of CMD, especially in Western and Nyanza provinces. All three begomoviruses (ACMV, EACMV, and a Kenyan isolate of EACMV-UG, EACMV-UG[KE]) were detected in samples from these two provinces, with many samples having a mixed infection of ACMV and EACMV-UG[KE]. Interestingly, mixed infections of EACMV and ACMV rarely were observed. The detection of EACMV in samples from the two provinces of Kenya confirms earlier work (8), which exposed the virus in a sample collected near Kisii in Nyanza province. In this study, EACMV was detected in four samples from the region, but the percentage of samples with the virus was very low comparable to those with EACMV-UG[KE] or ACMV. This clearly

shows that, despite earlier reports (12), the geographic distributions of EACMV and ACMV overlap. Although the presence of EACMV alone in the coastal areas of Kenya seems to present little problems to cassava production, ACMV and EACMV-UG[KE] threaten the survival of the crop in the western parts of the country.

Virus infections clearly were obtained after sap extracted from cassava plants showing abnormal symptoms not similar to those caused by CMD was rub inoculated onto *N. benthamiana* and cassava. Attempts to induce a double infection of the two viruses using sap extracted from plants with a mixed infection of a begomovirus and filamentous viruses in *N. benthamiana* or cassava were unsuccessful; instead, only filamentous viruses were transmitted, indicating that filamentous viruses have higher sap transmission efficiencies than begomoviruses. However, biolistic inoculation using DNA extracted from such plants resulted in only EACMV infections. This could be explained by the fact that DNA had been treated with RNase during one of the extraction stages destroying filamentous virus genomes. Moreover, it confirms that these viruses have RNA genomes. In cassava, the viruses induced symptoms of chlorotic spots that enlarged as the leaves

Fig. 2. A, *Nicotiana benthamiana* showing symptoms of yellow net and vein banding 10 days after sap inoculation. B, Sap-inoculated cassava showing symptoms of infection with cassava filamentous viruses. C, Reactions of *N. glutinosa* 24a after infection with cassava filamentous viruses. D, Severe mosaic and mottling symptoms on cassava infected with *East African cassava mosaic virus* and hitherto undescribed filamentous viruses.

aged, and irregular yellow vein banding that appeared on young leaves 2 to 3 weeks after sap inoculation. This contrasts sharply with the findings of Bock (1) on CBSV, in which symptoms were not that severe and were found only on old leaves of cassava plants. Despite repeated attempts, Bock failed to transmit the virus back to cassava by sap inoculation. This implies either that Bock was working with a different virus or that the cassava samples used in this study were infected with a virus that could be transmitted back to cassava in addition to CBSV. An additional argument against single infection of cassava with CBSV is Bock's description of symptoms as appearing only on older cassava leaves and not on both young and old leaves as observed in this study. Viruses of 650 nm in length were reported to be associated with cassava at the coastal region of Kenya (1) but the existence of filamentous viruses of shorter lengths never has been reported in that area. The presence of slightly flexuous rod-shaped particles equivalent in length to 650 nm imply a possible association of a carlavirus with

the infection. On the other hand, the presence of pinwheels and flexuous rod-shaped particles was an indication that a potyvirus is associated with the infection. Surprisingly, however, all the antisera used in ISEM for potyvirus identification failed to decorate the particles.

Based on SDS-PAGE analysis of crude sap and partially purified virus preparation, the capsid-protein sizes of 42 and 38 kDa were observed in all preparations while a 28-kDa band was observed in a few of them. The size of CPs of potexviruses has been reported to be in the range of 21 to 27 kDa (14), implying that the 28-kDa band observed in this study belongs to a virus either in the potexvirus group or to viruses that were described as undefined structures. The other possibility could be that either the 38- or 42-kDa band was degraded by proteolytic enzymes of plant or microbial origin (11), reducing its size to 28 kDa. Comparisons of the protein bands separated in the SDS-PAGE of partially purified virus preparations versus crude sap of virus-infected and noninfected plants revealed that the 54-kDa band is

most likely to be of host plant origin, whereas the 42-, 38-, and 28-kDa bands are of viral origin. This, however, could not be verified by Western blot analysis due to lack of virus-specific antisera. Furthermore, attempts to amplify potyvirus sequences using an RT-PCR approach with degenerate potyvirus genus-specific primers (5) were not successful. Thus, the identity of the virus or viruses in these cassava plants still remains elusive. Nevertheless, the results of back transmission experiments clearly demonstrated that these viruses are true pathogens of cassava. The scope of this work did not allow further characterization of these filamentous viruses; however, future work may concentrate on purification, antibody production, and genome analysis. In addition to virus characterization, identification of the viruses' vector or vectors and production of virus-free planting material are crucial topics for future work. This study has provided evidence that, other than begomoviruses that cause serious diseases of cassava in Africa, filamentous viruses also are available and, despite their limited distri-

Fig. 3. A, Electron micrograph showing a negatively stained (1% uranyl acetate) preparation of begomovirus (*East African cassava mosaic virus*) particles from crude sap extracted from cassava leaves. Bar represents 100 nm. B, Carlavirus-like particles in partially purified virus preparation from *Nicotiana glutinosa* 24a. Globular structures are contaminations of the preparation with phyto-ferritin. Bar represents 200 nm.

Fig. 4. Electron micrograph showing negatively stained preparations (from *Nicotiana benthamiana*): A, potyvirus-like particles, B, pinwheel structures, C, potexvirus-like structures, and D, undefined rod-shaped structures. Bar represents 500 nm.

Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of protein preparations of plants infected with cassava filamentous viruses. Lane 1 = *Gomphrena globosa*, 2 = *Nicotiana benthamiana*, 3 = *N. clelandii*, 4 = *N. debneyi*, 5 = *N. glutinosa* 24a, 8 = noninfected *N. debneyi*, lanes 6 and 7 = virus preparations, M = Marker. M_r (kDa).

bution, could reach local significance and can most probably be as serious as begomoviruses.

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