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Cassava latent virus specific DNAs in mosaic diseased cassava of Nigerian origin

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The mosaic disease of cassava (*Manihot esculenta* Crantz), prevalent in many parts of Africa, is perpetuated by vegetative propagation of diseased material and by transmission of the causal agent, African cassava mosaic virus (synonym of cassava latent virus, CLV), a geminivirus by the whitefly *Bemisia tabaci* (Genn.). Isolates of the virus from Kenya, Nigeria and Angola have been shown to be distinguishable, but related, in serological and nucleic acid hybridisation tests (Harrison, 1985). The complete nucleotide sequence of the two circular single-stranded (ss) DNA components, DNA 1 (2.78 kb) and DNA 2 (2.72 kb), of a Kenyan (K) strain of CLV is known (Stanley and Gay, 1983) and several double-stranded (ds) and ssDNA forms, isolated from the propagation host *Nicotiana benthamiana*, infected experimentally with the virus, have been characterised (Stanley and Townsend, 1985). In the present paper we report on the intracellular DNA forms, extracted directly from cassava plants, naturally infected with CLV, which have not been investigated previously.

Plants of a mosaic diseased cassava clone, Lagos-Agege N (LG1-N), and *N. benthamiana,* were cultivated as previously (Adejare and Coutts, 1982). Nucleic acid extracts from mosaic diseased cassava leaves, or from the leaves of *N. benthamiana* plants infected by mechanical inoculation with sap from mosaic diseased leaves (Adejare and Coutts, 1982) were prepared by the method of Hamilton et al. (1982). Virus-specific nucleic acid species were separated and detected, using methods described by Coutts and Buck (1985), by electrophoresis of the extracts through a 1% agarose gel, transfer to GeneScreen Plus membrane and hybridisation with [^{32}P)-labelled nick-translated plasmids pJS092 and pJS094 which contain CLV-K DNA 1 and DNA 2 cloned into the *Mlu* I and *Pst* I sites respectively of Ml3 vectors (Stanley, 1983). The results (Fig. 1) show that the CLV-specific nucleic acid species extracted from CLV-infected cassava and CLV-infected *N. benthamiana* are the same in number and electrophoretic mobility, but that they differ in their relative proportions.

Confirmation of the DNA nature of the nucleic acid species giving positive hybridisation signals (Fig. 1, track A, bands 1 to 5) was obtained following RNAse digestion of LG1-N cassava extracts prior to electrophoresis which resulted in no signal reduction on probing (Fig. 1, track B) while DNAse treatment abolished all signals (Fig. 1, track C). Furthermore S1 nuclease digestion of the extracts abolished the signals produced by bands 4 and 5 (results not shown) suggesting their ss conformation and a ds nature for bands 1, 2 and 3. All nuclease digestions were performed as previously (Hamilton et al., 1982).

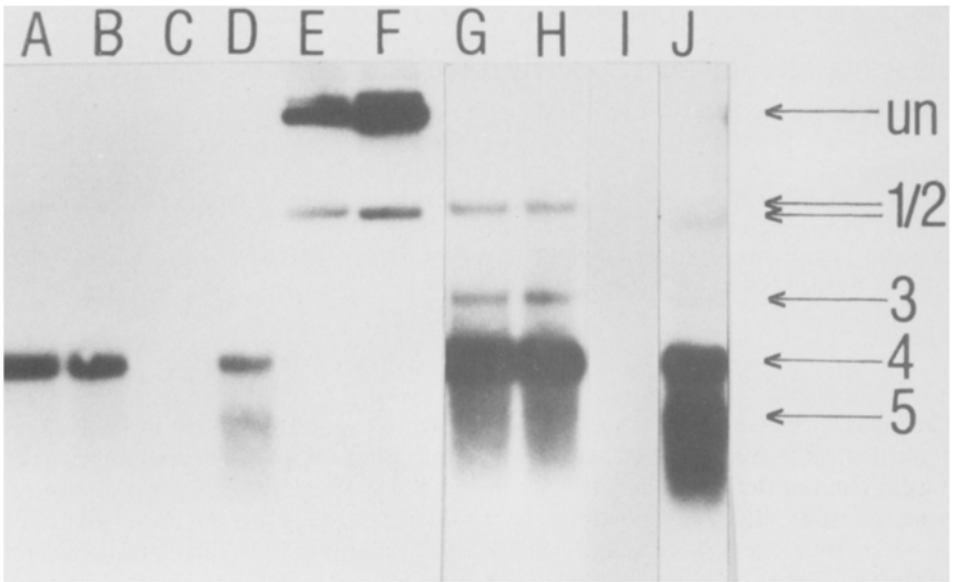

Fig. 1. Autoradiograph of Southern blotted gel containing nucleic acid extracts of mosaic-diseased cassava clone Lagos-Agege (LG1-N) (tracks A, B, C and G, H, I). *Nicotiana benthamiana* tissue infected with cassava latent virus (CLV) passaged from LG1-N (tracks D, J) and recombinant plasmid DNAs pJS092 (DNA 1) and pJS094 (DNA 2) restricted respectively with *Mlu* I (track E) and *Pst* I (track F). Hybridisation was with a nick-translated probe specific for a mixture of CLV-K DNAs 1 and 2. Tracks G, H, I and J represent longer exposure times of A, B, C, and D respectively. Extracts in tracks B and C were treated with RNAse and DNAse respectively prior to electrophoresis, as described in the text. Bands of interest are labelled 1 to 5 as described in the text; un refers to plasmid vector DNA.

The profile of virus-specific DNA forms present in extracts of LG1-N cassava and CLV-infected *N. benthamiana* tissue was similar to that observed previously for extracts derived from *N. benthamiana* infected with the closely related geminivirus, tomato golden mosaic virus (TGMV) (Hamilton et al., 1982). From comparative data and the nuclease digestions described above we deduce that in nucleic acid extracts of both cassava and tobacco tissue bands 1 and 2 represent respectively the open circular and linear ds forms of CLV DNA while band 3 is the supercoiled form of the dsDNA. Band 4 DNA is virion ssDNA since purified genomic DNA of another previously charactrised Nigerian isolate 631104 (Adejare and Coutts, 1982) co-migrated in gels (results not shown). A smaller, subgenomic ssDNA species, band 5 appeared to vary in amount in the different extracts. Following passage of the virus from LG1-N cassava to *N. benthamiana* much larger amounts of band 5 DNA were present in extracts of the latter as compared to the former (c.f. Fig. 1 tracks A, G, with D, J).

The band 5 ssDNA species illustrated in Fig. 1 (all tracks excepting E and F) are of a size and topology to be similar to subgenomic ssDNA species found in extracts of CLV-K infected *N. benthamiana* tissue. These subgenomic species are a population of similarly sized molecules (ca. 1.3 kb) derived from the smaller genomic DNA compo-

nent by specific deletions (Stanley and Townsend, 1985). The finding of subgenomic DNA in extracts of cassava naturally infected with CLV suggests that the CLV subgenomic DNA described by Stanley and Townsend (1985) arose by amplification in *N. benthamiana* of a subgenomic species already present in mosaic-diseased cassava, rather than its formation *de novo* in *N. benthamiana*. This suggestion is consistent with the observations that no subgenomic DNA was detected in *N. benthamiana* plants infected with cloned CLV DNA 1 and 2 or after five successive passages of the progeny virus (Stanley, 1983; Stanley and Townsend, 1985).

Following inoculation of *N. benthamiana* plants with mixtures of the cloned CLV-K DNA genomic components and the cloned subgenomic DNA, increasing the proportion of the subgenomic DNA in the inoculum rendered it progressively less infectious as judged by an increase in the time required for symptom development as well as the overall decrease in the number of plants which became infected (Stanley and Twonsend, 1985). Thus it has been suggested (Stanley, 1985; Stanley and Townsend, 1985) that the encapsidated CLV subgenomic DNA resemble defective-interfering (DI) particles associated with animal DNA virus infections (Holland, 1985). Bearing this suggestion in mind and our findings of only small amounts of subgenomic DNA in extracts of severely mosaic diseased cassava plants, it will be interesting to quantify the levels of this DNA in affected plants and correlate them with symptom severity. Of particular interest would be the reactions of strains or variants of CLV in different cassava clones raised under different growth regimes. Indeed the characteristic periodic incidence of symptoms of the disease in cassava may only reflect changing levels of the subgenomic DNA in the plants. This feature may be especially important in future resistance breeding programmes for cassava.

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Samenvatting

Voor 'cassava latent' virus specifieke DNA's in mozaïekzieke cassaveplanten afkomstig uit Nigeria

Het genoom van het 'cassava latent' virus (CLV), een geminivirus, bestaat uit twee cirkelvormige, enkelstrengige DNA-moleculen nl. DNA 1 (2,78 kb) en DNA 2 (2,72 kb). DNA, verkregen uit *Nicotiana benthamiana*-planten, die mechanisch waren geïnoculeerd met sap van natuurlijk geïnfecteerde cassaveplanten, bevat naast het enkelstrengige genoom-DNA en de corresponderende open, lineaire en via covalent-bindingen gesloten cirkelvormige, extra-getwiste, dubbelstrengige vormen, een enkelstrengig DNA, dat kleiner is dan het genoom-DNA (c. 1,3 kb). Van dit DNA is aangetoond, dat het fungeert als een defect DNA. Het kleinere DNA interfereert met de functies van het genoom-DNA waardoor de plant minder hevige ziektebeelden gaat vertonen. Het

was echter nog niet bekend of dezelfde DNA-vormen in het veld voorkomen in natuurlijk geïnfecteerde cassaveplanten met mozaïeksymptomen. In het hier beschreven onderzoek is aangetoond, dat de DNA-vormen in natuurlijk geïnfecteerde cassaveplanten overeenkomen met die in kunstmatig met het CLV geïnfecteerde *N. benthamiana*-planten. De hoeveelheid DNA, kleiner dan het genoom, wordt echter sterk verhoogd door passage van het virus van cassave naar *N. benthamiana*. De mogelijkheid, dat de hoeveelheid van het DNA, kleiner dan het genoom, in mozaïek-vertonende cassaveplanten gecorreleerd is met de mate van symptoomexpressie wordt besproken.

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