# Studying begomovirus infections in susceptible and resistant cassava breeding lines

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## Introduction

Screening for virus resistance in cassava in the field relies on natural infestations with whitefly vectors transmitting the particular virus(es) present at a given time and location, hence comprising several unknown factors. The evaluation of host resistance from a virological point of view is a comparison of defined characters, e.g. virus accumulation in resistant versus susceptible plants, hence:

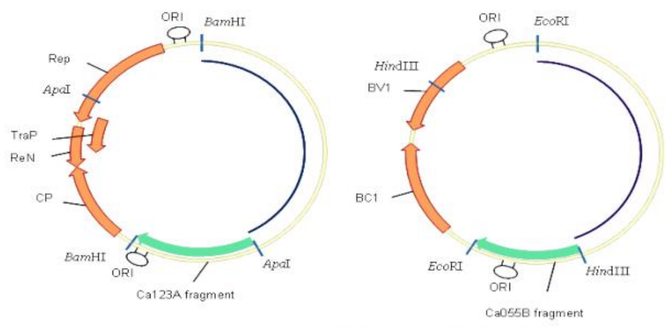
**We have established methods, to inoculate cassava with defined begomoviruses to study virus infections in resistant and susceptible cassava breeding lines.**

## Viruses, constructs and inoculations

Partial, head-to-tail dimers of DNA-A and DNA-B genomic components were constructed for virus species/ strains of *African cassava mosaic virus*, ACMV [NG] and ACMV[ KE], and for *East African cassava mosaic virus*, EACMV [KE] and EACMV-UG2 [KE].

For biolistic inoculation, total DNA from infected plants (2µg/shot) or mixtures of begomovirus cloned DNA-A and DNA-B multimers (0.5µgA/0.5µgB/shot) were introduced into cassava by microprojectile bombardment of DNA coated onto 1 micron gold particles. Biolistic inoculation was done with the Helios gene gun at 200-300 psi applying 2-3 shots/ plant.

Grafting experiments were performed for verification and reference and to study virus movement/accumulation in resistant/susceptible branches.




Partial multimeric constructs of cloned DNA-A and DNA-B genomic components of begomoviruses to reach high infection rates in cassava. DNA-A and DNA-B mixes from heterologous viruses form pseudo-recombinant viruses.

## Quantification of virus accumulation

A real time PCR was developed for quantitation of virus in infected plants. SYBR Green I or Taq-Man fluorescence was measured. 18S DNA sequences were used as endogenous control for normalisation.

## Cassava breeding lines

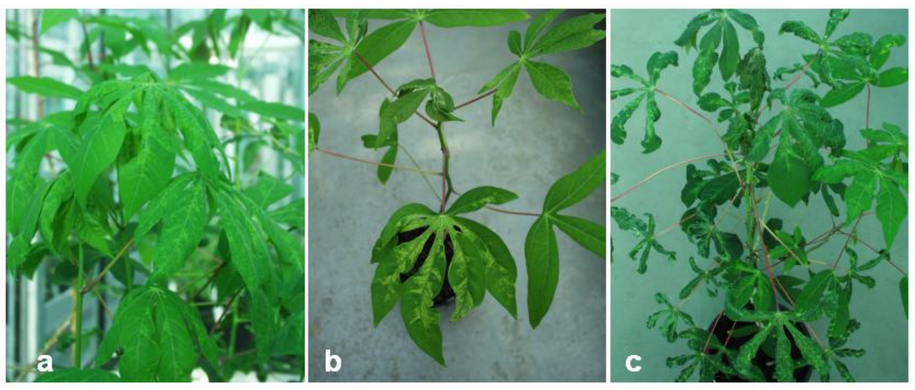
Cassava breeding lines and local landraces with varied resistance status were subjected to virus inoculation experiments

| Accession | Source/locality | Parents | Resistance to CMD |
|---|---|---|---|
| ***TME 117*** | SW Nigeria | Unknown | HS |
| ***TME 3*** | SW Nigeria | Unknown | HR |
| ***TME 4*** | SW Nigeria | Unknown | HR |
| ***96/1089A*** | IITA Nigeria | M94/0461 * / 90/01554 | **HR** |
| ***96/1039*** | IITA Nigeria | M85/00680 */ 90/01554 | **HS** |
| ***96/0304*** | IITA Nigeria | 91/02327 * / M94/0461 | **HS** |
| ***96/0160*** | IITA Nigeria | 30572 * Atu | **HR** |

HS=highly susceptible, HR=highly resistant

## Begomovirus infections in cassava

### in TME 117, *susceptible,* - 96/0304, 96/1039




**Virus infections introduced by microprojectile bombardment,**
**a)** EACMV-UG2 [KE] DNA-A/B; **b)** EACMV-[KE] DNA-A/B **c)** EACMV-[KE] DNA-A/B / ACMV [NG]

Inoculations with cloned viruses resembled wild type infections (a,b).

Mixed virus infections resulted in serious disease phenotypes (c).

EACMV-UG2 [KE] Ca55-A/B

EACMV-[KE-Kilifi] CA123A /EACMV-UG2 Ca055 B

Inoculations with chimerical virus clones resulted in severe disease phenotypes

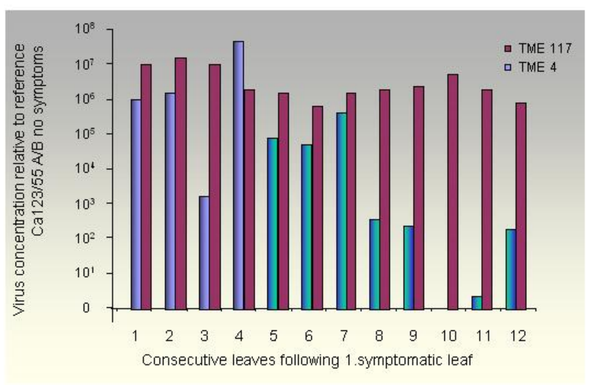
### in TME 4, *resistant,* - TME 3, 96/1089A, 96/0160


**Virus infections introduced by microprojectile bombardment,**
a) cloned EACMV-UG2[KE] Ca55-A/B; b) cloned EACMV-[KE-Kilifi] CA123A / EACMV-UG2 Ca055 B; c) Cutting from recovered symptomless, TME4 grafted on EACMV [KE-Kwale] **infected rootstock.**

Inoculations with cloned homologous components of viruses did not establish infections (a). Inoculation with pseudorecombinant virus (b) resulted in virus infections with severe symptoms on 2-4 leaves followed by recovery. Graft-inoculation of EACMV-UG2 to TME 4 resulted in virus invasion, symptom expression and recovery. Cuttings excised from completely recovered plant parts (c) were still carrying virus, supporting replication, symptom expression and recovery.

## Evaluation of virus accumulation in resistant cassava

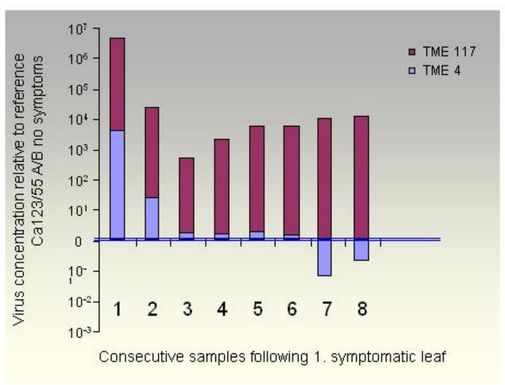
EACMV-[KE-Kilifi] CA123A /EACMV-UG2 Ca055 B




In **TME 117** a high, relatively constant virus load is maintained.

In **TME 4,** symptomatic leaves (1-4) show comparable virus concentrations, non-symptomatic **leaves** have variable virus content, ranging from absence of virus (10-11) to $10^5$ times the virus positive baseline reference.

ACMV/EACMV-UG2 graft inoculation




In ACMV/EACMV-UG2 graft inoculated branches of TME117 and TME4, the TME4 branch recovers from symptoms and virus is still barely detected.

Virus concentration is only a fraction of susceptible TME 117.

## Conclusions

- Inoculation by microprojectile bombardment of begomoviruses (cloned or total DNA) resulted in infections in susceptible cassava breeding lines;
- Upon inoculations with chimerical virus clones, a pseudorecombinant virus EACMV-UG/EACMV-UG2 is formed with severe symptom phenotypes in susceptible and resistant cassava varieties; Similarly, mixed virus infections result in serious virus synergism.
- Cassava lines with resistance against CMD, proved resistant against all begomoviruses tested;
- In resistant cassava, virus replication is supported for a limited time and subsequently aborted, leading to plants recovered from symptoms and virus.
- However evidence is pending whether resistant cassava recovers free of virus!