Pathogenicity of infectious clones of cassava mosaic viruses and potential for their use to screen cassava genotypes for disease resistance

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Introduction

The pathogenicity of a virus determines the effects on growth and crop yield and hence the losses it causes. Therefore establishing infectivity to analyse pathogenicity determinants is key in devising control strategies to diseases caused by viruses. Cassava mosaic disease (CMD) remains the main constraint to cassava production in many areas, where it causes huge economic losses. The use of resistant varieties is the most practical method for control. However, lack of data on infectivity and pathogenicity associated with causal viruses is one of the factors limiting the screening and use of resistant varieties. This study has determined the genetic diversity of CMD-associated geminiviruses in Uganda, assessed their infectivity and used infectious cloned genomic components of cassava mosaic geminiviruses (CMGs) to screen cassava varieties for resistance to CMD.

Fig 1: Genomic organization of bipartite begomoviruses

Materials and methods

Genetic diversity was assessed by PCR-RFLP and sequence analysis. Full-length clones were isolated and their infectivity confirmed by biolistic inoculation of *Nicotiana benthamiana*, cassava and *Manihot glaziovii* Muell.Arg. Pathogenicity ratings were based on symptom severity (in a highly susceptible cassava variety, Ebwanateraka: Scale 1-5; 1= Symptomless, 5= Very severe chlorosis leaf distortion and general stunting) and virus accumulation determined by probe-hybridization. Virus clones were used to screen cassava germplasm for resistance to CMD. The contribution of viral genes in disease development was analysed by site-directed mutagenesis.

Results

• Diversity of CMD-associated geminiviruses has been determined (Figure 2 a,b,c).

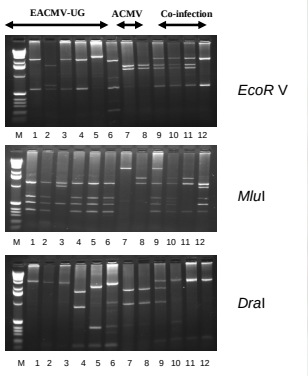

Fig 2a: RFLP profiles.

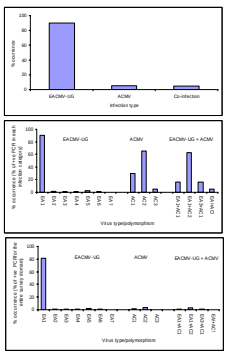

Fig 2b: Occurrence of CMD-associated viruses/combinations.

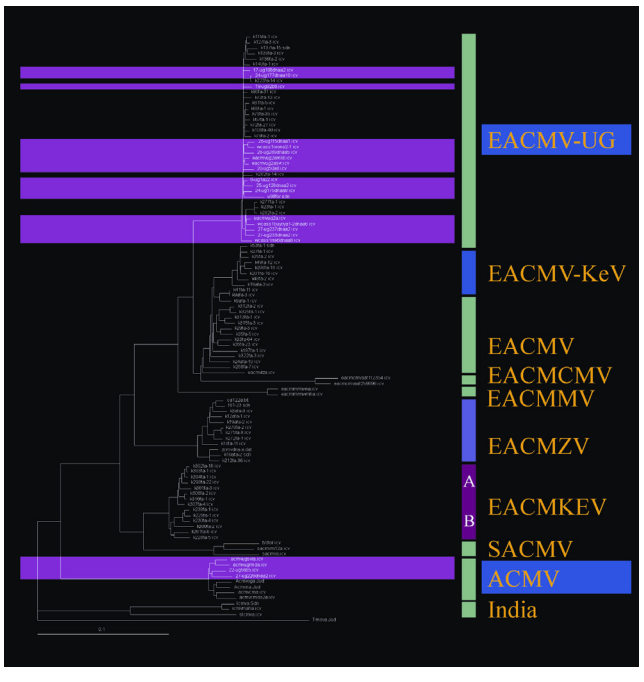

Fig 2c: Phylogenetic analysis shows relatedness of DNA A sequences of cloned viruses (pink).

• Pathogenic variation of strains and species associated with CMD in Uganda has been demonstrated (Figure 3).

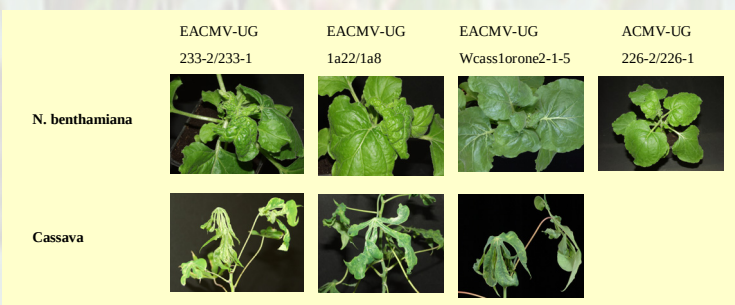

Fig 3: Symptoms induced by different EACMV-UG & ACMV isolates in *N.benthamiana* and cassava.

• Infectivity is greatly influenced by the type of virus-virus interaction (Figure 4).
• Synergism between ACMV and EACMV-UG is associated with severe symptoms (Figure 4a).

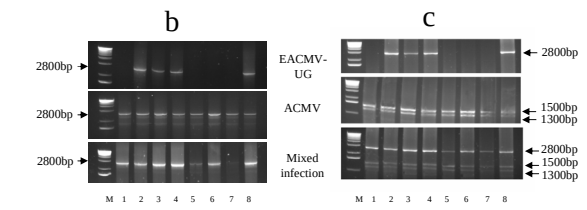

Fig 4a: Symptoms induced by ACMV (A), EACMV-UG (C) and their combination (B) [a]. Analysis of progeny virus by PCR [b] and *EcoR* V restriction digestion of universal primer PCR products, showing both viruses in the progeny virus of each infected plant of the mixed infection [c].

• Differences in the ability of EACMV-UG isolates to interact synergistically with ACMV has been established.
• The contribution of viral genomic components to synergism has been established.
• The effect of intra-species co-infections on disease development has been assessed.
• The contribution of viral genes *AV2*, *AV1*, *AC2* in disease development in *N. benthamiana* and cassava has been established.
• Differences in response of different cassava varieties to inoculation with virus clones has been established, which provides an opportunity to screen cassava germplasm for resistance to CMD (Figure 5).

Severity score	**5**	**5**	**3**	**3**	**1**	**1**
Infected/inoculated	**3/3**	**3/3**	**2/3**	**1/3**	**0/3**	**0/3**
Resistance factor	**500VA1**	**500VA2**	**200VA3**	**100VA4**	**0**	**0**

Ebwanateraka | Njule Omumyufu | Njule Omweru | Nase 4 | TME 14 | Nase 12

Fig 5: Symptoms induced by an EACMV-UG clone in a different cassava varieties showing differences in response to inoculation. PCR analysis of total DNA from TME 14 and Nase 12 did not reveal systemic infection. Resistance rating factor equals severity score x incidence x virus accumulation index (VA).

Discussion

This study has demonstrated pathogenic variation within Ugandan CMD-associated geminiviruses. The contribution of intra- and inter-species virus interactions to pathogenicity has been assessed and is being further evaluated by site-directed mutagenesis to delineate contributory sequences. The interactions between viruses lead to different effects on growth and yield and can have serious effects on resistance deployed. The use of infectious clones offers an opportunity to screen for durable resistance to single or mixed virus infections as a complementary approach to the conventional screening procedures against a diverse range of CMGs. Further evaluations are focusing on establishing whether the current resistance is specific or broad spectrum to CMGs prevalent in the region.

Acknowledgements

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