# NOVEL AMPELOVIRUSES INFECTING CASSAVA IN THE CENTRAL AFRICA AND THE SOUTH-WEST INDIAN OCEAN ISLANDS


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## BACKGROUND

- Cassava is one of the most important food crops in Africa and its production is seriously damaged by viral diseases.
- Two devastating diseases of cassava, cassava brown streak disease and cassava mosaic disease, are caused by viruses. For eight years now, no new virus have been reported on this crop.
- Identification of viral agents responsible of diseases in plants is a key step for establishing diagnostic tools and developing control strategies.

## METHODS

- Leaf samples from plants of different landraces were collected from farmer fields and from the germplasm collections of the Conseil Départemental, of CIRAD and of FOFIFA between 2015 and 2017.
- Total RNA, double-stranded RNAs (dsRNAs) and virions were extracted and purified from leaves
- High throughput sequencing (HTS) was performed following virion-associated nucleic acid (VANA), dsRNAs as well as total RNA strategies and target.
- Bioinformatic analyses were performed using CLC Genomic Workbench and Geneious.
- Sequence presence were confirmed by RT-PCR
- Defective molecules were sanger-sequenced using pJET1.2

## RESULTS

- Thirteen viral sequences were characterized in field-grown cassava plants from the Democratic Republic of Congo, Madagascar, Mayotte and Reunion Islands
- Phylogenetic trees and the average aminoacid divergence rates well over the 25% species demarcation criterion for two of the three proteins (RdRp and HSP70h) supports the existence of two distinct species tentatively named Manihot esculenta-associated virus 1 and 2 (MEaV-1 and MEaV-2).

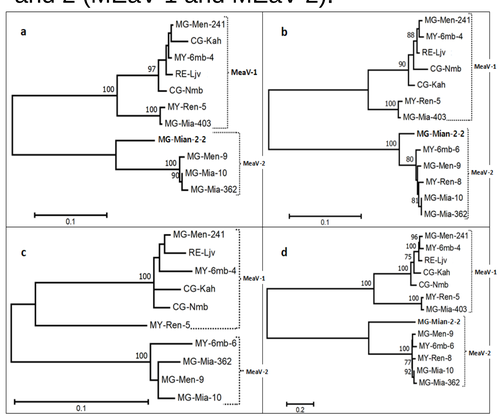



**Fig. 1.** Phylogenetic trees reconstructed using the amino acid sequences of the three taxonomically relevant proteins for the family *Closteroviridae*: (**a**) RdRp; (**b**) HSP70h; (**c**) CP and the whole genome nucleotide sequences (**d**)

- Sequence analysis (length ranging between 10,417 and 13,752 nucleotides) revealed seven open reading frames.
- The replication-associated polyproteins have the three expected functional domains: methyltransferase, helicase and RdRp.
- Additional open reading frames code for a small transmembrane protein, a heat-shock protein 70 homolog, a heat shock protein 90 homolog, a major and a minor coat protein.
- Reads belonging to four defective RNAs molecules were identified in accessions from Reunion Island and from Madagascar. Deletions were confirmed by targeted RT-PCR and sequencing. Deleted zones cover different genome regions.

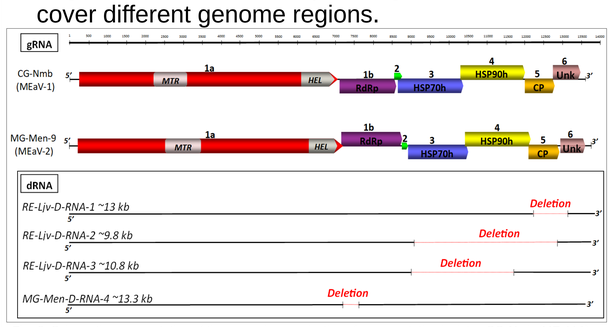



**Fig. 2.** Schematic representation of the genomic organization of representative isolates CG-Nmb (MEaV-1) and MG-Men-9 (MEaV-2) (top) and structure of the defective variants (**dRNA**) identified (bottom)

- Phylogenetic analyses showed that MEaV-1 and MEaV-2 belongs to the family *Closteroviridae*, genus *Ampelovirus*, and in particular to its subgroup II

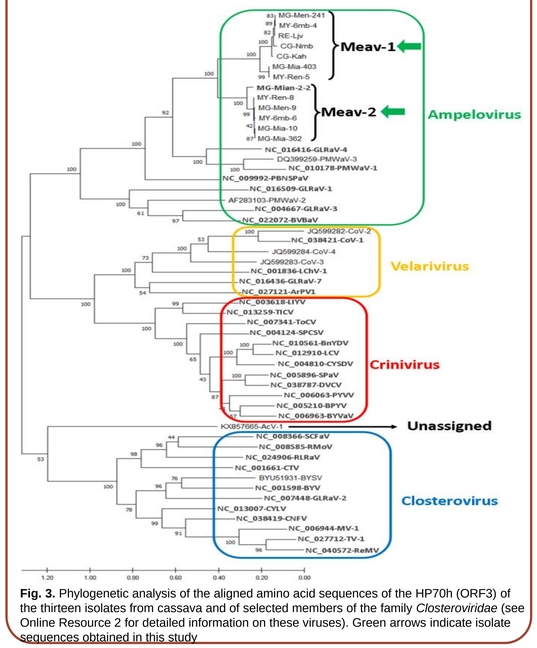
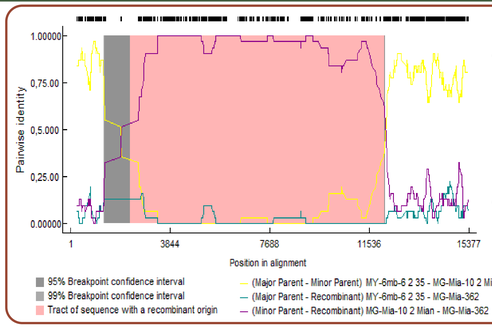
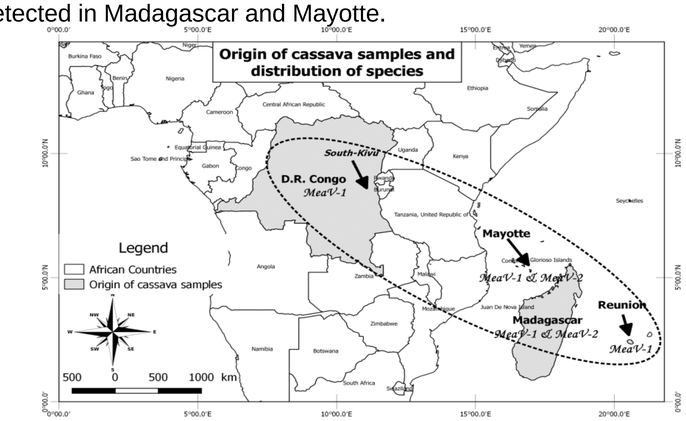



**Fig. 3.** Phylogenetic analysis of the aligned amino acid sequences of the HP70h (ORF3) of the thirteen isolates from cassava and of selected members of the family *Closteroviridae* (see Online Resource 2 for detailed information on these viruses). Green arrows indicate isolate sequences obtained in this study




- Recombination event involving MEaV-2 isolates from Madagascar (2 isolates) and from Mayotte (1 isolate) is reported.
- No evidence of recombination between MEaV-1 and MEaV-2 was detected.

MEaV-1 was found in all the countries of study while MEaV-2 was only detected in Madagascar and Mayotte.




## CONCLUSION & PERSPECTIVES

- This new ampelovirus complex has already a wide geographical distribution. This could be due to the lack of clear association with symptoms that could favor the multiplication of plant through cuttings from infected but asymptomatic plants.
- Association with symptoms as well as the impact on yield are under investigation.
- Investigations are conducted to estimate the synergistic interaction between coinfecting viruses (cassava brown streak viruses and cassava mosaic geminiviruses).
- Studies are needed to assess the impact of defective molecules on the epidemiology and pathogenicity of these new agents.
- Additional experiments are needed to characterize in depth the biology of the virus and the associated phytosanitary risks on wide scales.
- There is a need to develop diagnostic test in order to be able to evaluate the distribution and prevalence of these new viral agents in other regions of the world and evaluate their impact on the yield.