

Exploration profonde sur l'étiologie des maladies virales du manioc à l'Est de la R.D. Congo

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Project iCARE (Improved Cassava Virus Resistance mitigation strategies and development of a disease-free seed system)

Promotors: Prof. Espoir Bisimwa, Prof. Herve Vanderschuren,
Prof. Claude Bragard, Prof. Sébastien MASSART

PRESENTATION PLAN

- I. INTRODUCTION
 - I. General context
 - II. Specific context
 - III. Basic observations
 - IV. Research questions
 - V. Research objectives
- II. Study 1:
 - I. Methodology
 - II. Results
- III. Study 2:
 - I. Methodology
 - II. Results
- IV. Perspectives





Cassava plant:

- The third most important source of calories in the tropics (after rice and maize)
- A staple food for more than 800 million people, mostly in Africa.
- Africa is the highest producing continent & D.R. Congo is the 2nd highest producing country in Africa.



INTRODUCTION: Specific Context

Cassava: « THE ROOT OF THE PROBLEM »



1. Cassava mosaic disease: 11 sp. of ss-DNA viruses (CMBs).
2. Cassava brown streak disease: 2 sp. of ss-RNA viruses (CBSIs).



INTRODUCTION: Specific Context

Cassava: « **THE ROOT OF THE PROBLEM** »

+ 100 insects and mites, ~ 30 diseases induced by viruses, phytoplasmas, bacteria or fungi

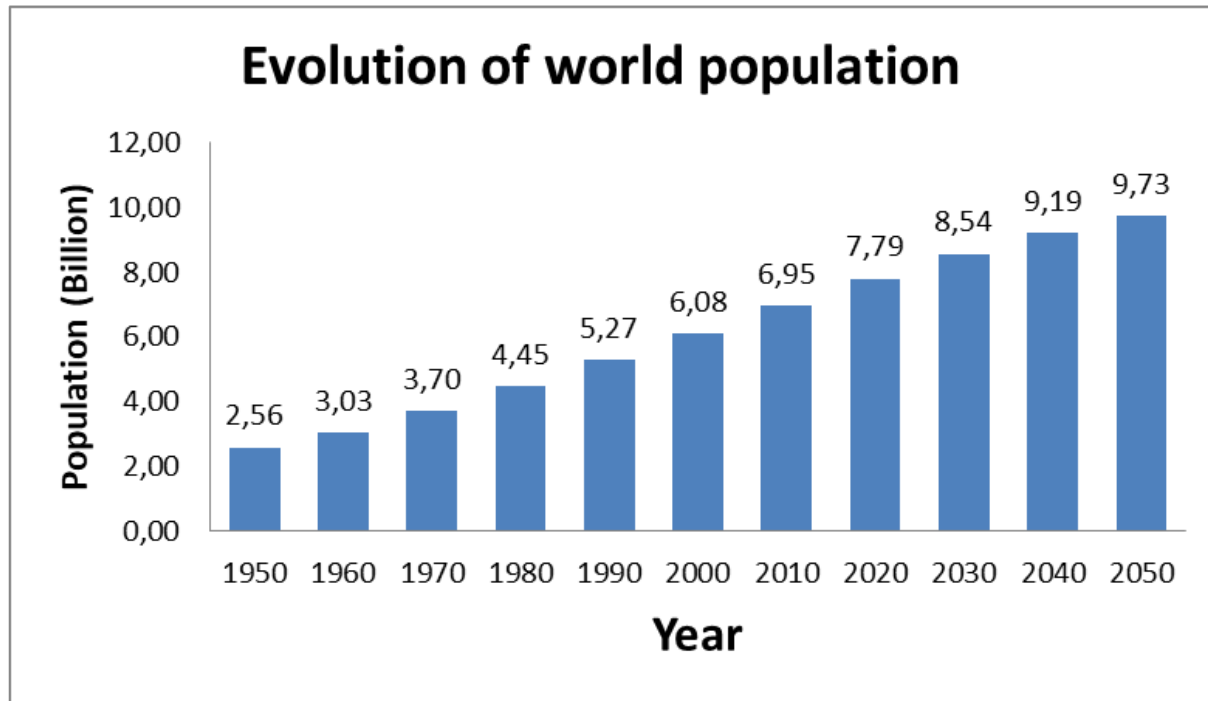


1. Cassava mosaic disease: \$1.2-2.4 billion annual loss
2. Cassava brown streak disease: \$726 million annual loss



INTRODUCTION: General Context

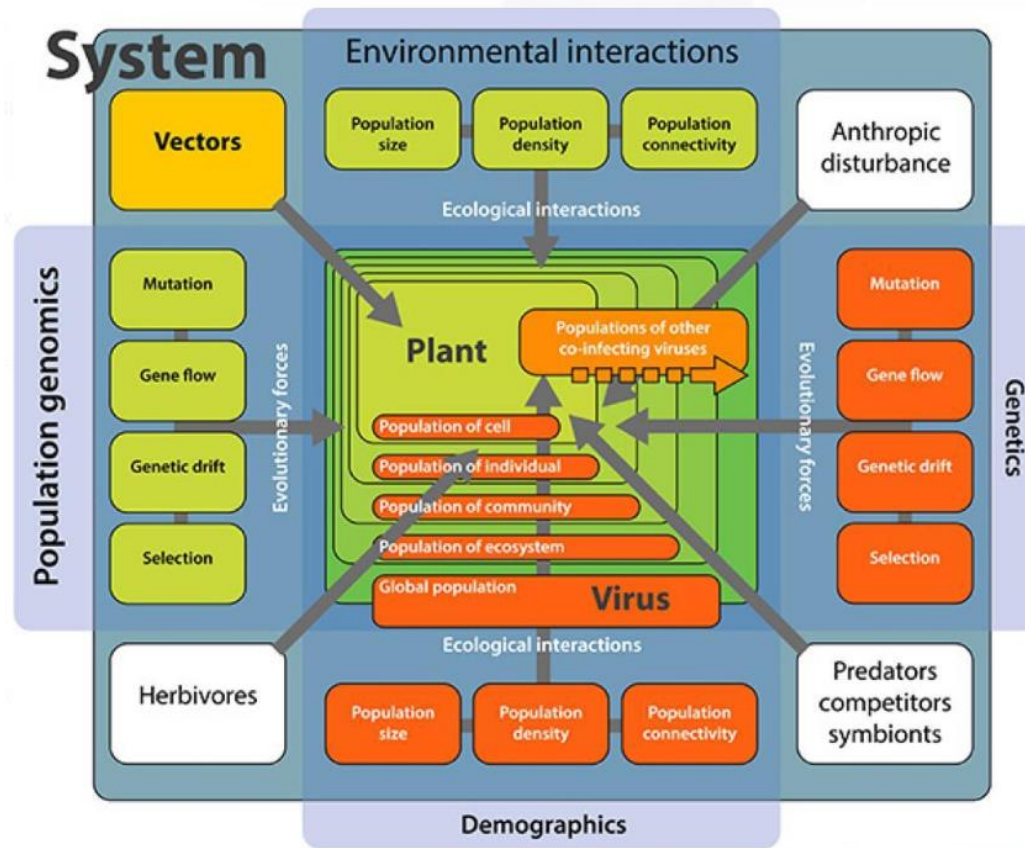
1. The world is undergoing a period of rapid expansion in human activity accompanied by an accelerating climate change.



2. Producing enough food, in a sustainable manner, to meet the needs of an increasing global population is one of the greatest challenges we face.

INTRODUCTION: General Context

3. Changes are impacting on plants, vectors & viruses causing increasing instability within virus-plant pathosystems.



4. Implications regarding our ability to achieve effective control of virus epidemics that diminish food production, especially those associated with virus emergence

INTRODUCTION: Specific Context

1. Viral genomes can evolve rapidly, sometimes leading to new diseases completely different. Reconstructing their genomes can advance the understanding and capabilities to combat the diseases they cause.
2. Wild flora acts as a reservoir of viruses causing significant losses in nearby crops and vice versa. However, information about viruses in wild species is still quite limited.

INTRODUCTION: Basic observations

Observ. 1: Although timing is not regular, depending on biological events and conditions (area under cultivation and climatic factors), major new diseases or strains of cassava disease tend to appear every 7–10 years !!!

Observ. 2: These two diseases are spreaded by whitefly vectors (*Bemisia tabaci*) and by the movement of planting materials

Question 1. Emergence of new disease every 7-10 years:

- Beyond what is actually known, are there other viruses waiting their time ?
- What can we learn by exploring viruses in wild species plants grown in cassava agrosystems ?



Question 2. Movement of planting materials

Between 80 and 90 percent of the seed that farmer's access comes from the local seed system.

➔ Which channels of cutting supply could be at risk of spreading viruses ?

INTRODUCTION: Research objectives

Study 1 : Cassava brown streak disease in the farmer's seed systems of the Eastern D.R. Congo: a cross-sectional understanding of risk factors associated with virus dissemination through seed channels/sources

➤ Objectives of the study:

1. Diversity of viral agents of CBSD & epidemic profile
2. Integrated approach (epidemic parameters+molecular detection findings+seed system parameters) → a deep understanding
3. Predict risk factors associated to the presence of CBSVs in fields grown with cuttings originated from diverse channels .



Study 2 :

Title: High throughput sequencing elucidate viral population in cassava agrosystems of the Eastern D.R. Congo

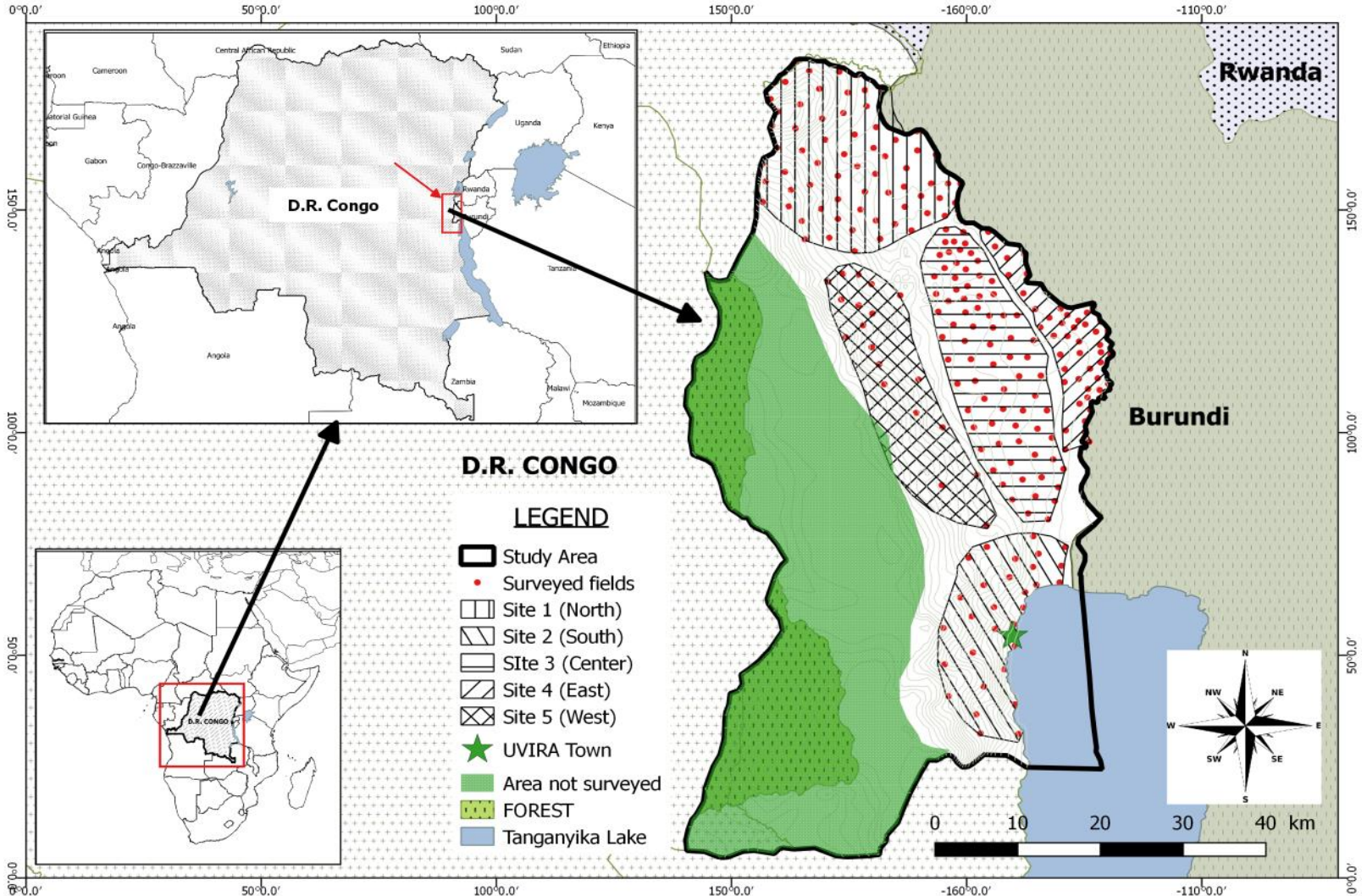
- Objective of the study: Characterize the diversity of viral population in :
 - ❖ Cassava plant
 - ❖ Weed plants



METHODOLOGY: Study 1

Title: CBSD in farmer's seed systems of the Eastern D.R. Congo: risk factors associated with virus dissemination through seed channels

1. Study area, survey & sampling: 65 villages, 246 fields



METHODOLOGY: Study 1

Title: CBSD in farmer's seed systems of the Eastern D.R. Congo: risk factors associated with virus dissemination through seed channels

2. Molecular detection: RNA extraction, RT-PCR
3. Data analysis: R & Quantum GIS
 - Statistical averaging & means comparison
 - Multivariate analyses: FAMD, HCPC
 - Binary logistic regression
 - GIS

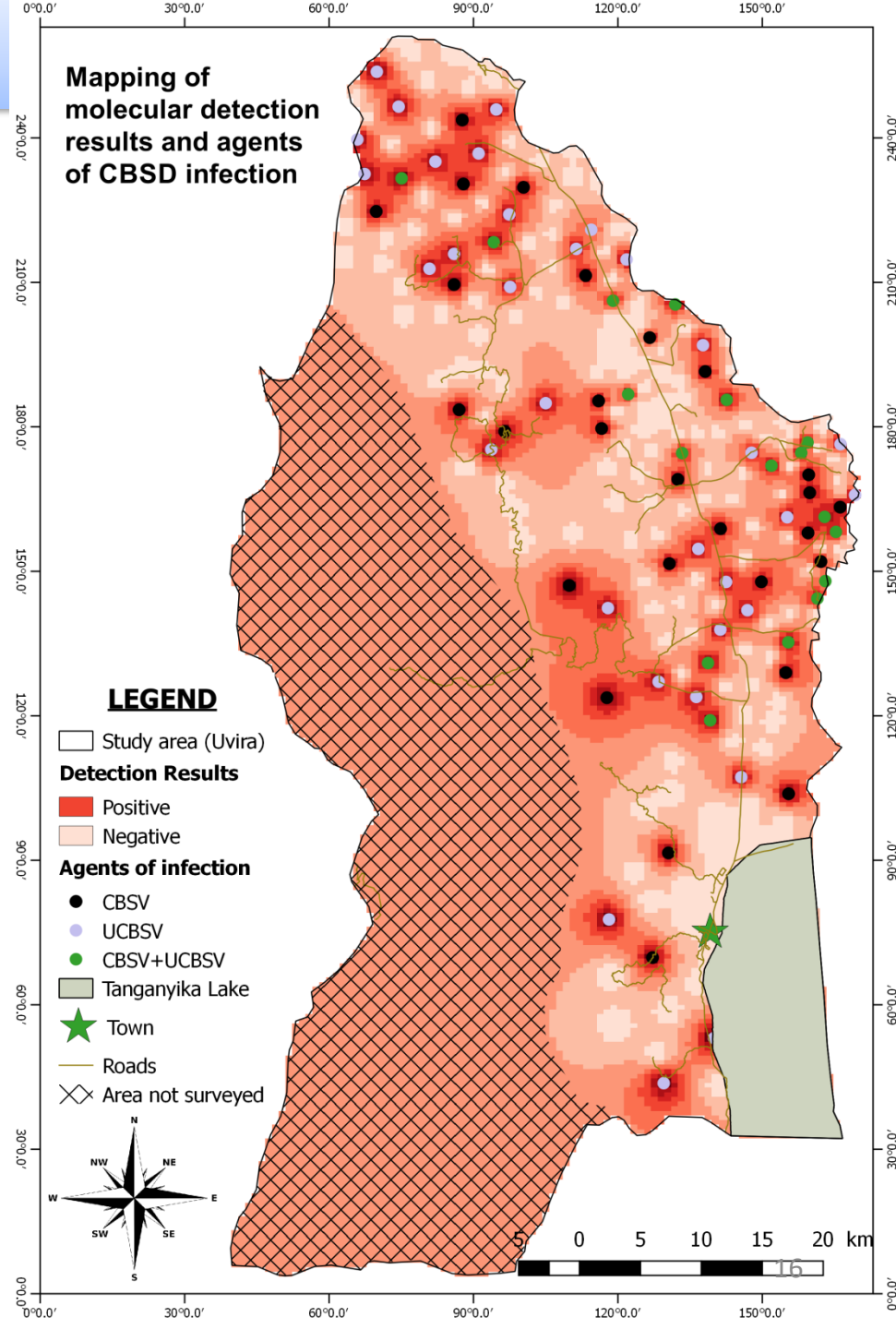


RESULTS: Study 1

1. Obj. 1: Epidemiologic aspects

Fig. Spatial trends of molecular detection findings (positive and negative samples) as well as the repartition of viral agents and types of infection (CBSV and UCBSV in single and dual infections).

- CBSV: uniform distribution in the whole study area
- UCBSV: North, Center & East
- CBSV+UCBSV: East
- Negative: South & West



RESULTS: Study 1

2. Obj. 2: Integrated description of the study area

Classification parameters	Zones		
	Cluster 1	Cluster 2	Cluster 3
Molecular detection	Negative	CBSV+UCBSV	UCBSV
Symptoms incidence	25% < 47%	41,8% < 47%	73% > 47%
Sites	2 and 5	1,3 and 4	1,3 and 4
Cutting channels	LF&CG, LF&NC	NC	LF & CG

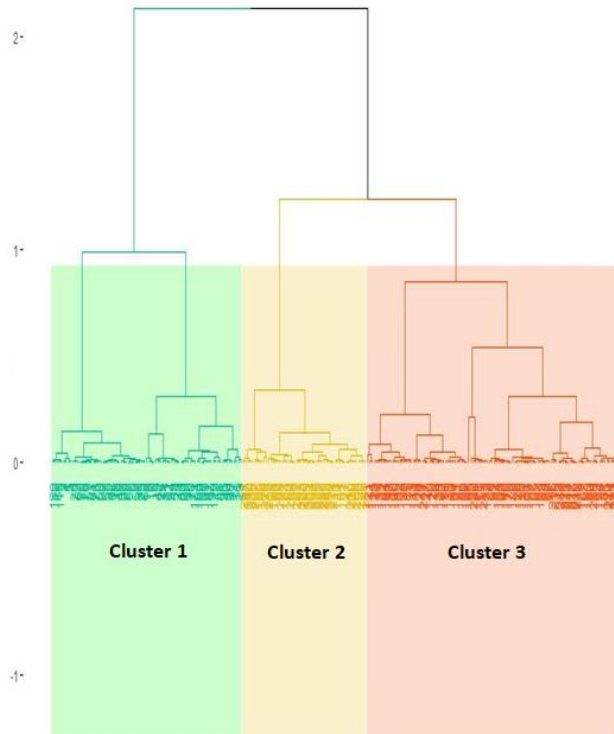
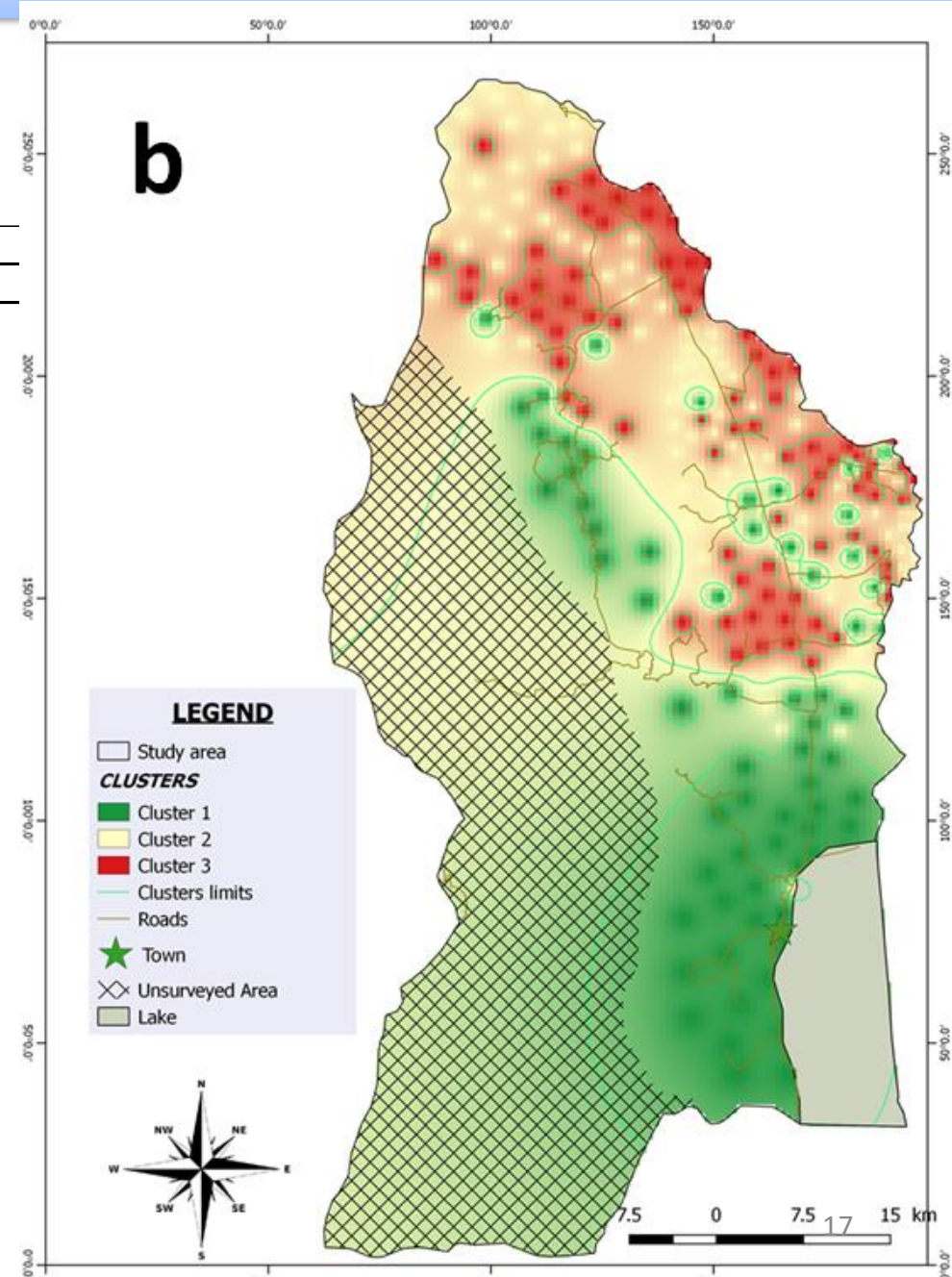


Fig. HCPC Cluster dendrogram



METHODOLOGY: Study 2

High throughput sequencing & viral population in cassava agrosystems

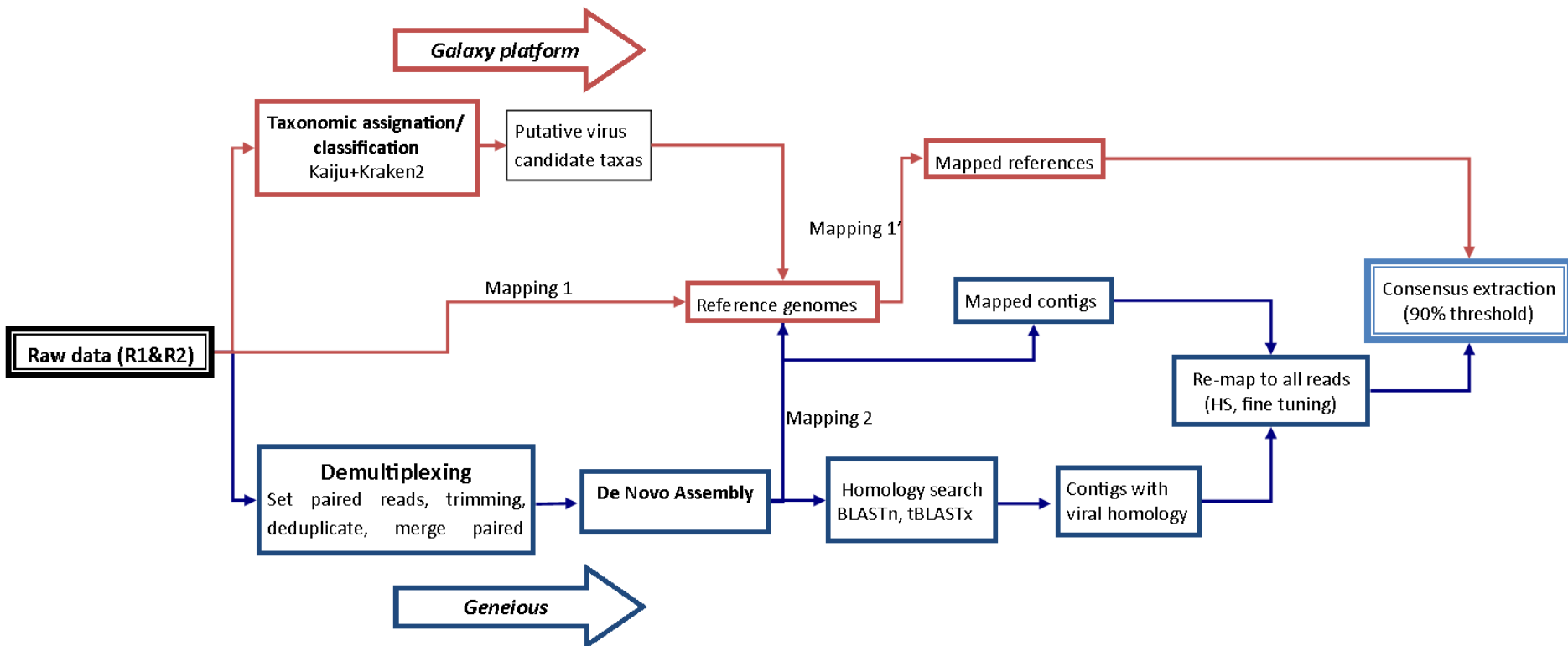
1. Origin of samples and sampling strategies: sampling from study 1.
 1. Cassava samples (leaves)
 2. Weeds
2. High throughput sequencing protocol:
 - Total RNA extraction: CTAB protocol, DNase treatment,
 - Library preparation: equimolar pooling (2500ng) of 7 samples (10 samples for weeds) → 14 pools in total
 - Sequencing strategy: Illumina TrueSeq Stranded total RNA



METHODOLOGY: Study 2

High throughput sequencing & viral population in cassava agrosystems

3. Bioinformatic analyzes: Discover new viruses **VS** detect known viruses



4. Sequence confirmation & gap-filling: RT-PCR and Sanger



RESULTS: Study 2

1. Obj. 1: Diversity of viral population in **cassava** plant

Table. Viral taxas identified in cassava plants

N°	Family>Genus	Species	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14
1	Potyviridae>Potyvirus	CBSV														
		UCBSV														
2	Geminiviridae>Begomovirus	ACMV														
		EACMV														
3	Begomovirus satellites	II														
		III														
4	Closteroviridae>Ampelovirus	MEaV-1														
5	Botourmiaviridae>Ourmiavirus	CV-C														
6	Secoviridae>Cheravirus	SPV														



RESULTS: Study 2

2. 1. Obj. 2: Diversity of viral population in **weed** plant

Table. Viral taxa identified in Weed plants

N°	Family>Genus	Species	P12	P13	P14
1	Luteoviridae>Polerovirus	BWYV			
		CCSV			
2	Luteoviridae>Enamovirus	Pepper enamovirus			
3	Endornaviridae>AlphaEndornavirus	Phaseolus vulgaris endornavirus-1(PVE-1)			
		PVE-2			
4	Potyviridae>Potyvirus	Potato virus Y (PVY)			
		Sun flower ring blotch virus			
5	Secoviridae>Cheravirus	SPV			
6	Caulimoviridae>Solendovirus	Tobacco vein clearing virus (TVCV)			
7	Tymoviridae>Tymovirus	Turnip yellows virus (TYV)			



RESULTS: Study 2

Two most important findings: new virus species detected in cassava plant:

1. Closteroviridae>Ampelovirus
2. Secoviridae>Cheravirus

Archives of Virology

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

Authors : Yves Kwibuka^{1*}, Espoir Bisimwa², Arnaud Blouin¹, Claude Bragard³, Thierry Candresse⁴, Chantal Faure⁴, Denis Filloux^{5, 10}, Jean-Michel Lett⁶, François Maclot¹, Armelle Marais⁴, Santatra Ravelomanantsoa⁷, Sara Shakir⁸, Hervé Vanderschuren^{8,9} and Sébastien Massart¹

RESULTS: Study 2

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

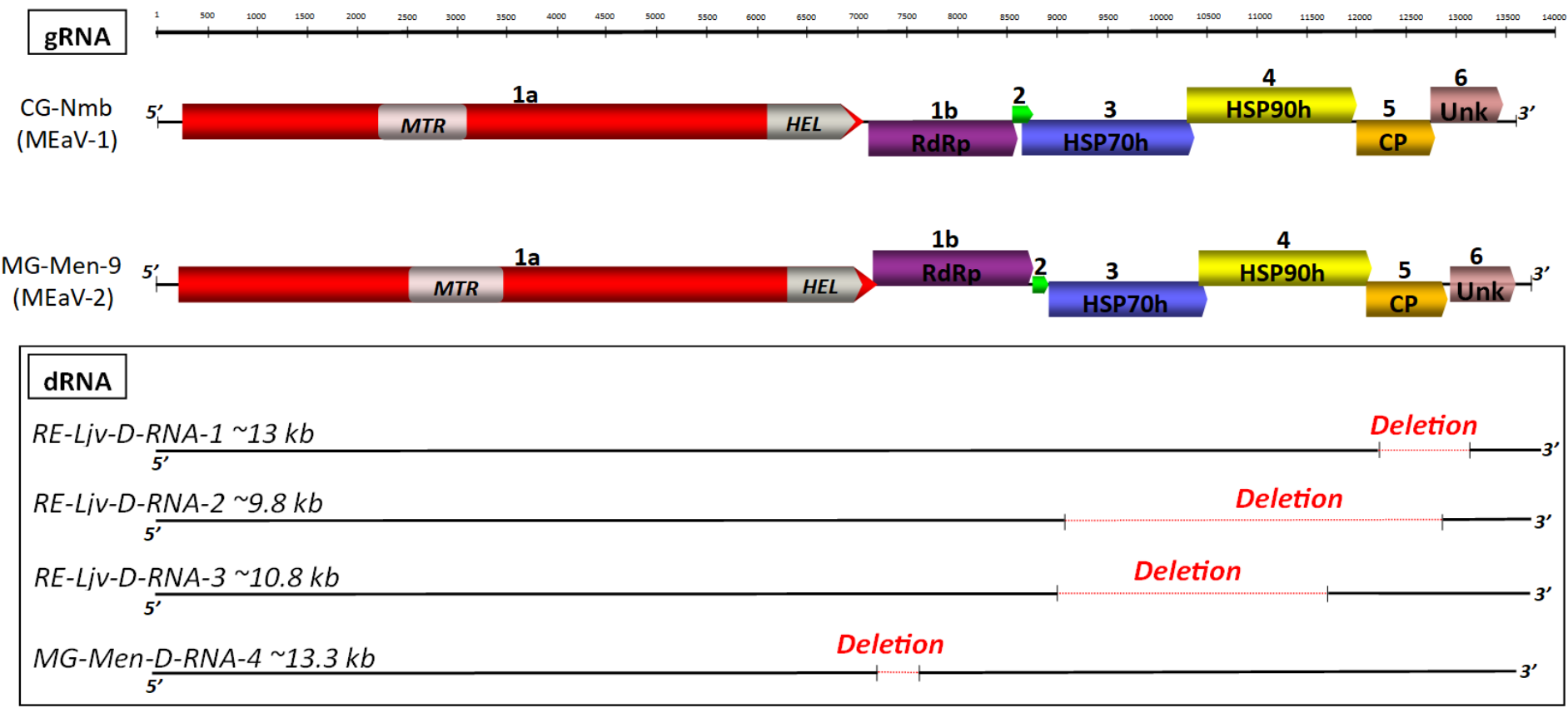


Fig. Schematic representation of the genomic organization of representative isolates CG-Nmb (MEaV-1) and MG-Men-9 (MEaV-2) (top) and structure of the defective variants (dRNA) identified (bottom).

RESULTS: Study 2

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

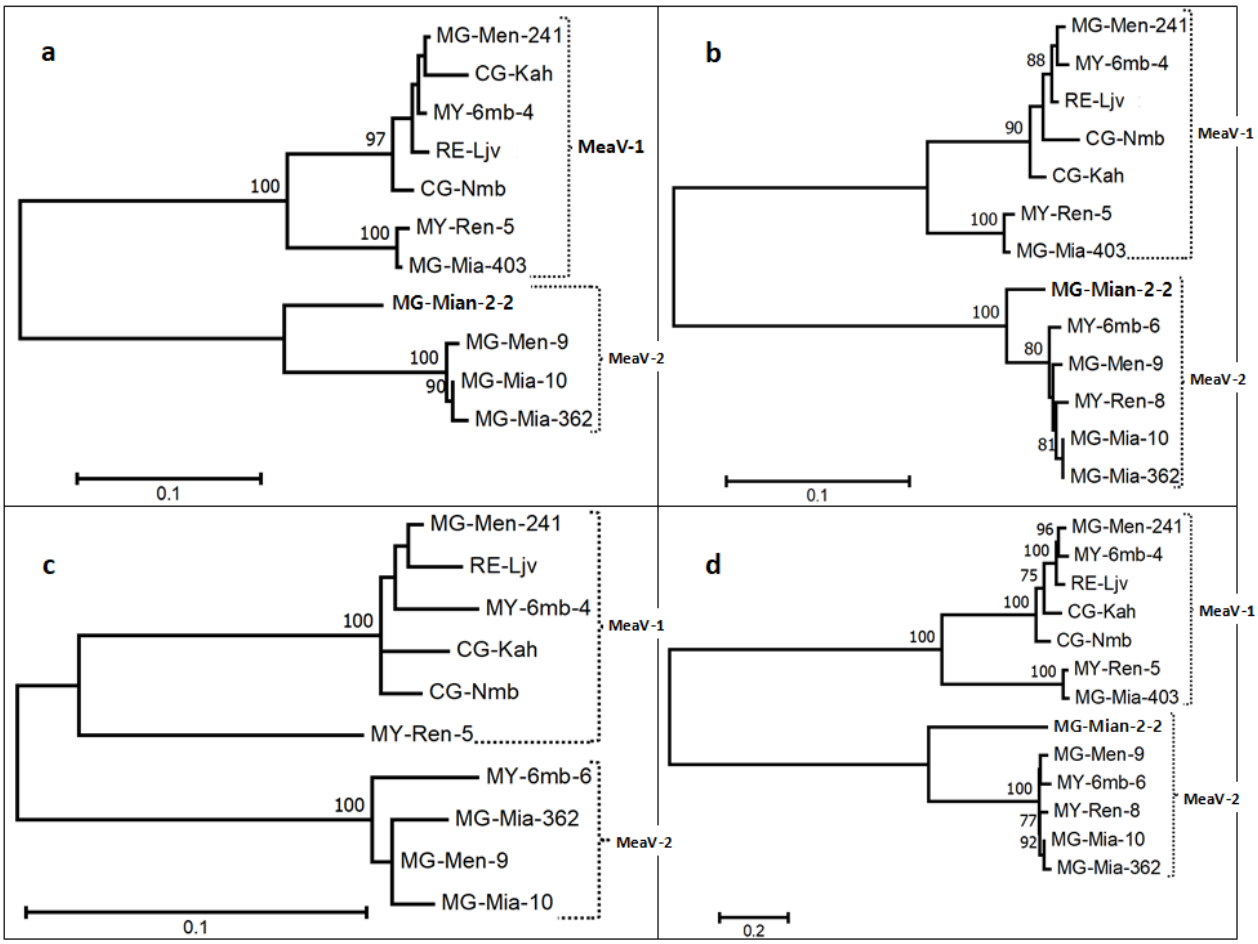


Fig. 3 Phylogenetic trees reconstructed using the amino acid sequences of the three taxonomically relevant proteins for the family *Closteroviridae*: (a) RdRp; (b) HSP70h; (c) CP and the whole genome nucleotide sequences (d). ML, GTR+GI model for nt, Poisson model for aa.

RESULTS: Study 2

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

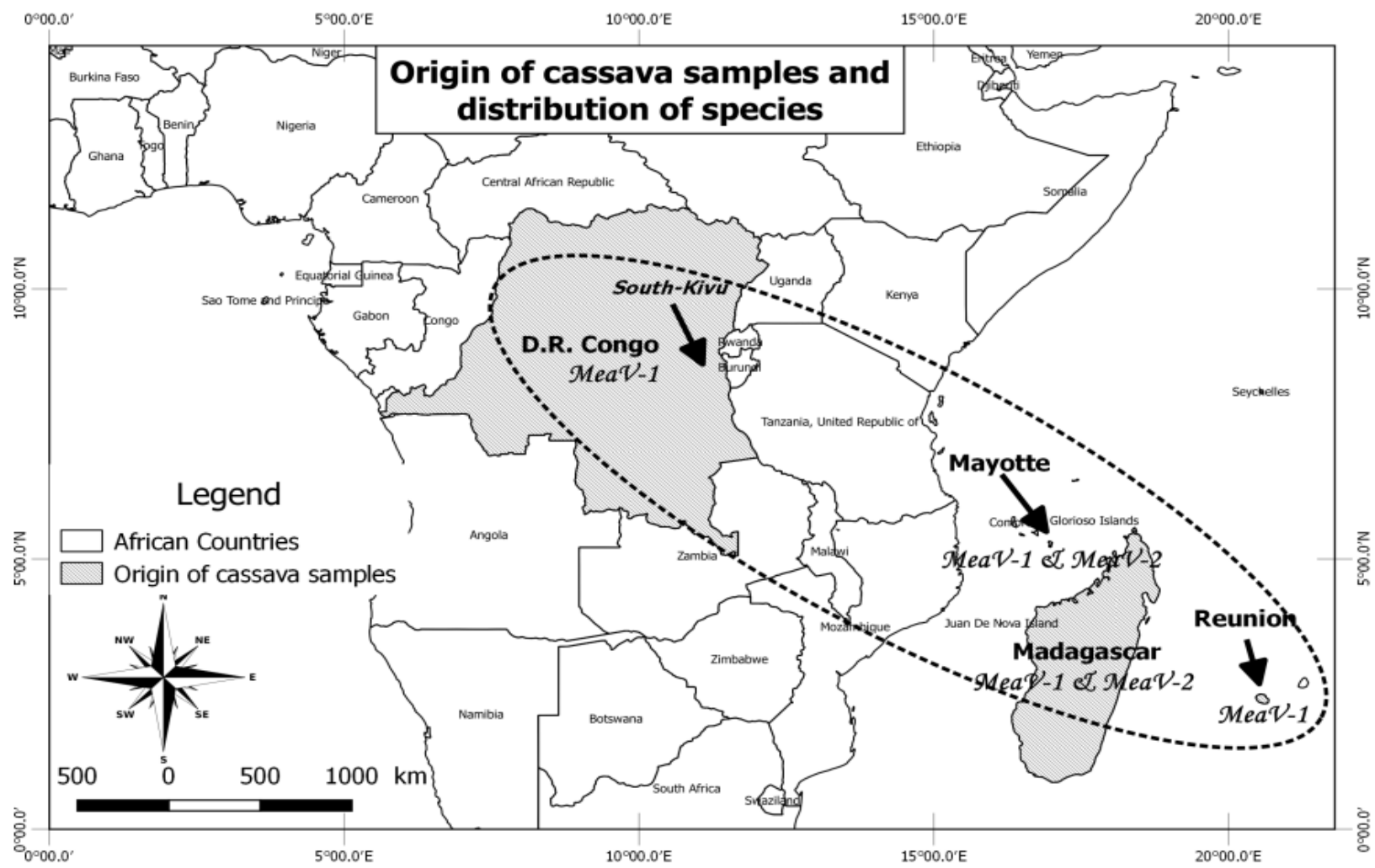


Fig. Geographic repartition of the two novel cassava ampelovirus species (MEaV-1 and MEaV-2) according to the country of origin of positive samples

RESULTS: Study 2

Family *Secoviridae* > Genus *Cheravirus* > Stocky prune virus

Cheravirus (cherry rasp leaf virus)



- Distinguishing features
 - Bipartite genomes, 3 capsid proteins of similar sizes, transmitted by nematodes (*Xiphinema americanum*) and through seeds
- Host range
 - ✓ Is broad or narrow, depending on the virus, and includes weeds in the vicinity of infected crops.
 - ✓ Symptoms are usually mild or absent
- Species demarcation criteria:
 - i. CP aa sequence < 75% identity
 - ii. Replication block aa < 80% identity



RESULTS: Study 2 Family *Secoviridae* >Genus *Cheravirus*>Stocky prune virus

Table. RdRp aa divergence

	Contig_26 _P3	Contig_83 P12	Contig_84 P12	Contig_7 P5	Contig_56 P4	Contig_107 P4	Contig_937 _P4	Currant_latent _virus	Apple_latent_s pherical_virus	Cherry_rasp_ leaf_virus	Arracacha _virus_B	Stocky_prune _virus
Contig_26_P3												
Contig_83P12	6,1%											
Contig_84P12	6,1%	0,0%										
Contig_7P5	3,7%	6,1%	6,1%									
Contig_56P4	3,7%	6,1%	6,1%	0,0%								
Contig_107P4	3,7%	6,1%	6,1%	0,0%	0,0%							
Contig_937_P4	3,7%	6,1%	6,1%	0,0%	0,0%	0,0%						
Currant_latent_virus	56,0%	55,0%	55,0%	55,0%	55,0%	55,0%	55,0%					
Apple_latent_spherical_virus	57,3%	56,3%	56,3%	56,5%	56,5%	56,5%	56,5%	24,7%				
Cherry_rasp_leaf_virus	55,7%	55,7%	55,7%	54,9%	54,9%	54,9%	54,9%	28,2%	29,4%			
Arracacha_virus_B	50,3%	50,3%	50,3%	50,0%	50,0%	50,0%	50,0%	43,2%	44,2%	44,5%		
Stocky_prune_virus	36,9%	36,2%	36,2%	35,2%	35,2%	35,2%	35,2%	49,7%	49,7%	50,3%	45,7%	
Contig_152P4	4,6%	7,4%	7,4%	0,0%	0,0%	0,0%	0,0%	52,6%	51,6%	50,9%	50,2%	35,2%

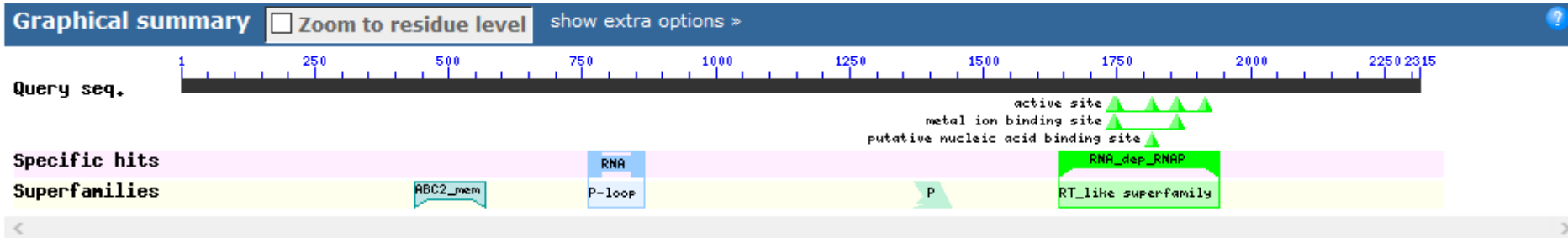
Divergence far below the sp. demarcation criteria in genus Cheravirus

Table. CP aa divergence

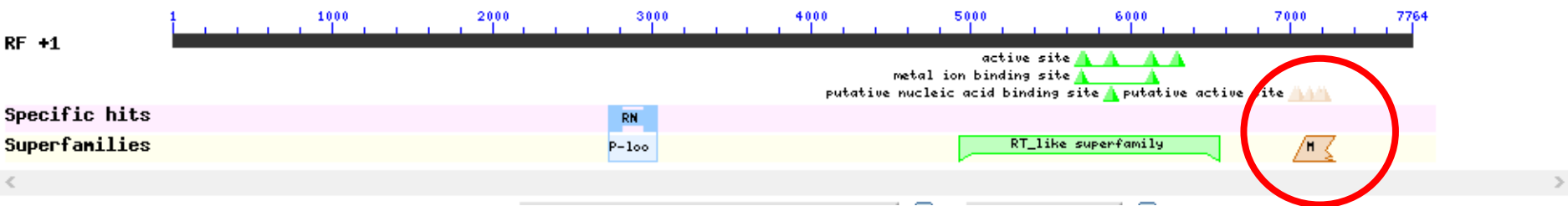
	Contig_926_ P12	Contig_2011_R NA_2_P12	Contig_2012_ RNA_2_P12	Currant_latent _virus	Apple_latent_s pherical_virus	Cherry_rasp_ leaf_virus	Arracacha _virus_B	Stocky_prune _virus	Contig_97_ P3_RNA2	Contig_80_ RNA2_P5	Contig_919_ RNA_2_P4
Contig_926_P12											
Contig_2011_RNA_2_P12	0,023161										
Contig_2012_RNA_2_P12	0,023161	0,006353									
Currant_latent_virus	0,735673	0,765557	0,761216								
Apple_latent_spherical_virus	0,762850	0,794798	0,794798	0,355932							
Cherry_rasp_leaf_virus	0,758782	0,790462	0,790462	0,395767	0,297782						
Arracacha_virus_B	0,802850	0,825581	0,824128	0,791418	0,807101	0,787843					
Stocky_prune_virus	0,699571	0,698376	0,700696	0,814385	0,807339	0,799076	0,834532				
Contig_97_P3	0,050797	0,099483	0,099483	0,737430	0,765625	0,760626	0,810286	0,711297			
Contig_80_P5	0,055664	0,098191	0,098191	0,738547	0,767857	0,762864	0,809143	0,713389	0,007656		
Contig_919_RNA_2_P4	0,109529	0,177979	0,177979	0,738095	0,764115	0,762203	0,807990	0,762162	0,056745	0,048218	27
Contig_1397_P4	0,056641	0,098191	0,098191	0,738547	0,767857	0,762864	0,809143	0,713389	0,008612	0,000939	0,047170

Presence of a MAF-HAM1 motif !!!

Arracacha virus B

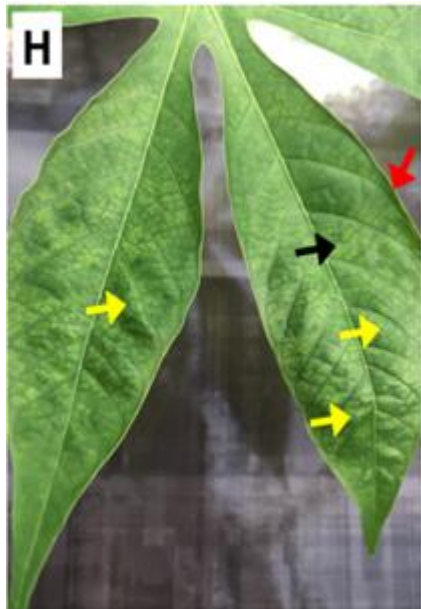


Our sequences



RESEARCH PERSPECTIVES

1. Confirmation of HTS results : RT-PCR
2. Biological aspects of Ampelovirus & Cheravirus:



Infected plants:

- Cheravirus only
- Cheravirus+UCBSV
- Ampelovirus+UCBSV

Healthy, susceptible scions

Var. 60444

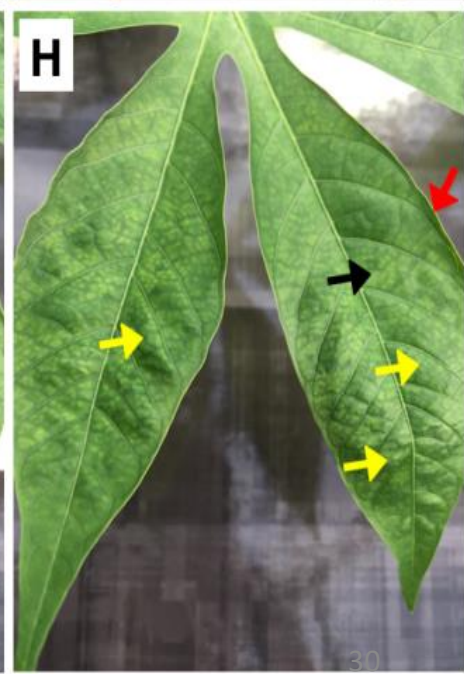
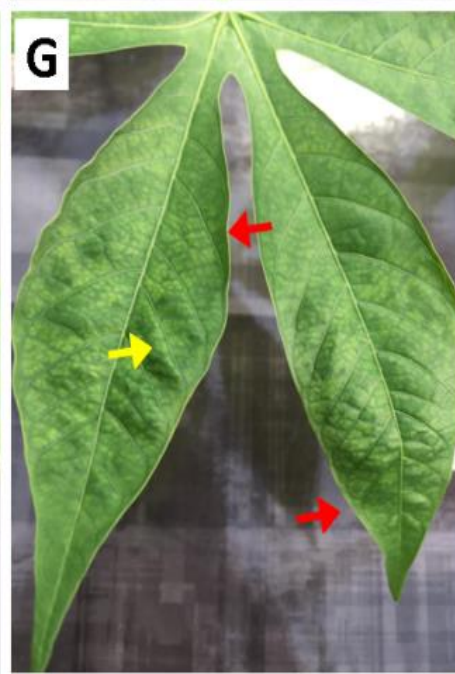
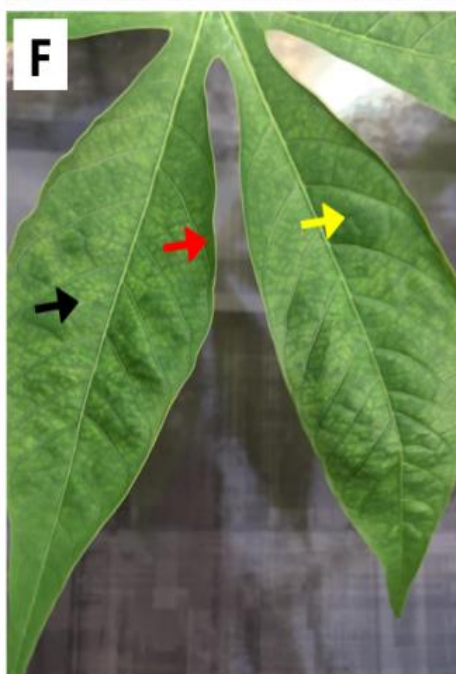
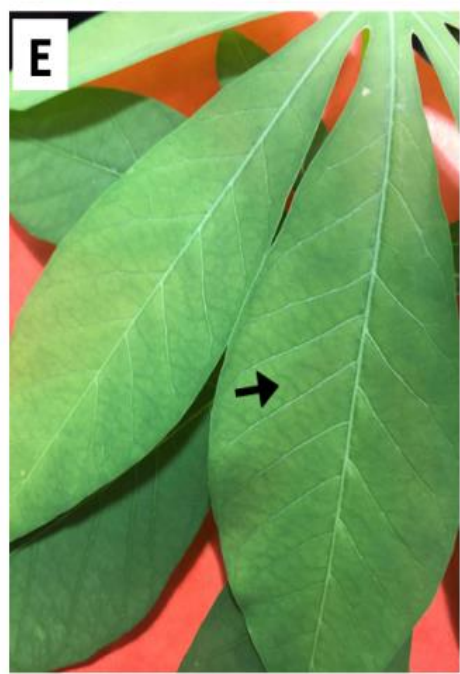


Infected rootstocks

- Symptoms characterization
- Virus translocation inside the plant

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End...