Pre-emptive disease management

Crop Protection

Cassava Program Retreat - Colombia

February - 2023

Crop Protection Team

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CWBD

Juan Manuel: CWBD in SEA first response and early observations

Warren A: Disease biology

Ana Maria: Bioinformatics

CBB

Juan Manuel/Rafael R: Models for resistance screening

CFSD –Jenyfer/Wilmer (10mins)

CBSD – Xiaofei Zhang (10mins)

Q&A (20mins)

Some notes on microbes

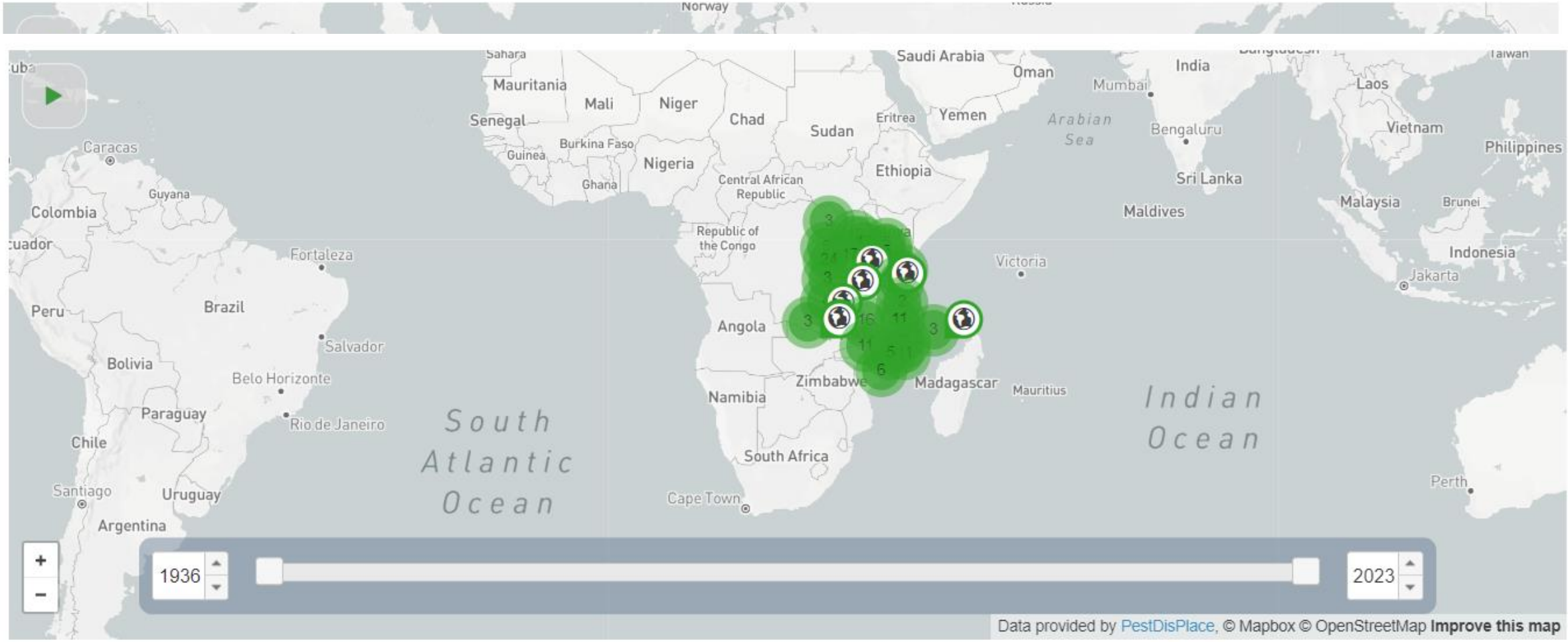
- Globally they are not equally distributed, most do not cause disease
- Those that do are professional party spoliers – evolving and moving
- We need pathogen genomic information to correctly identified them
- Is field diagnostics something to go for? (Nothing beats PCR yet)
- Going beyond first cases – study the dissemination scenarios (Geo location, vectors, climate)

Monitoring platform
https://pestdisplace.org

CMD in Asia – last 15 years

CFSD – last 50 years

CBSD – last 80 years

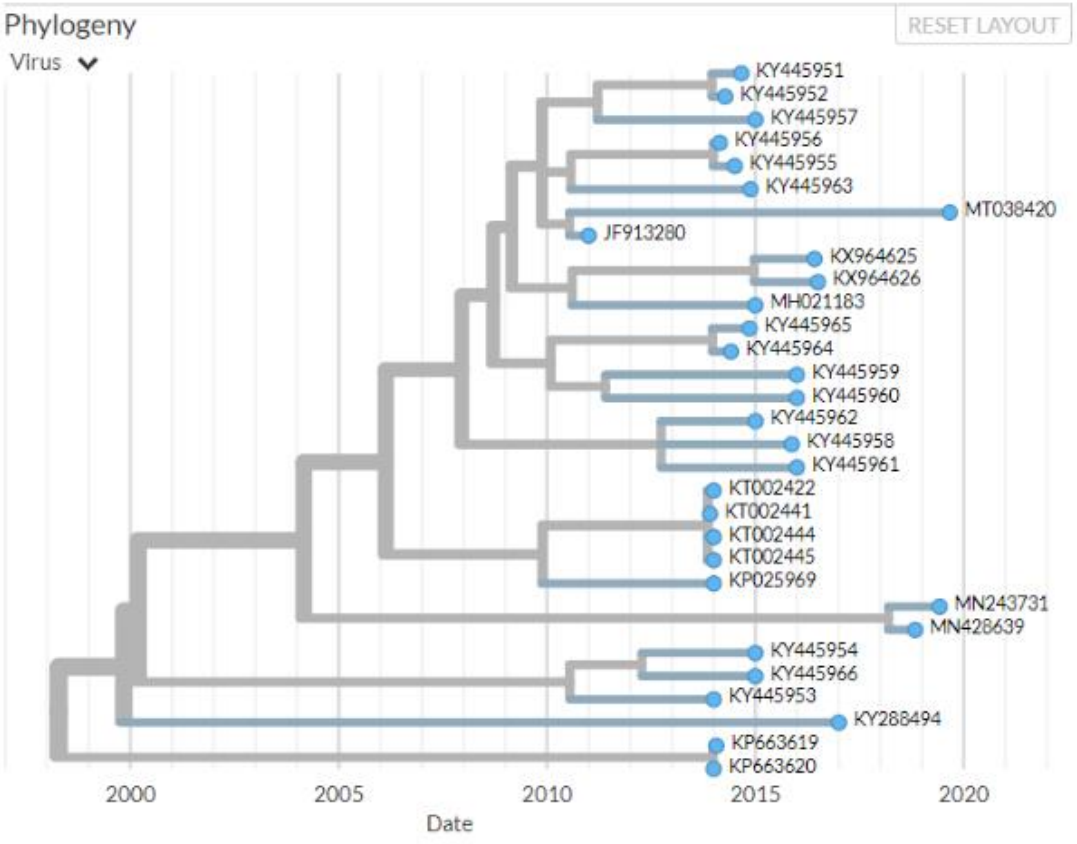


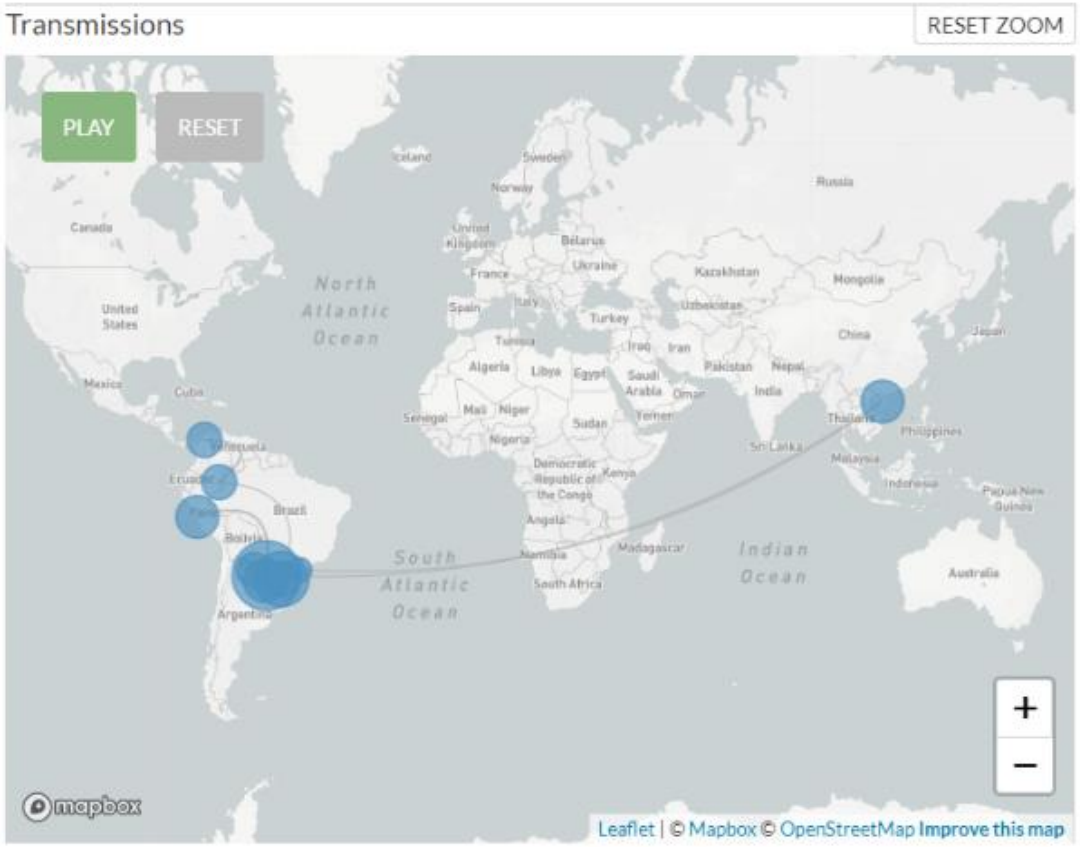
Cassava common mosaic disease – last 3 years

Real-time tracking of cassava common mosaic virus (RdRp gene)

Maintained by PestDisPlace.

Showing 31 of 31 genomes sampled between Dec 2010 and Sep 2019.





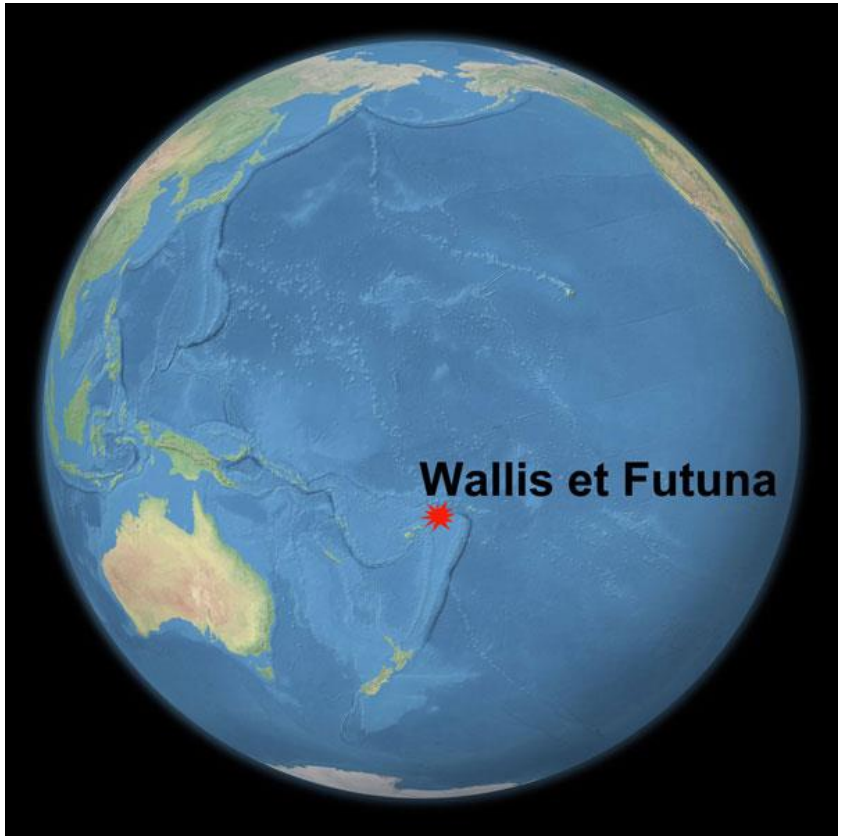
TR4 – last 15 years

Cassava Witches' Broom Disease (CWBD)

Juan M. Pardo, Warren Arinaitwe, Ana M. Leiva

The CWB disease begin….

In May 2004, a survey for plant diseases caused by viruses and virus-like pathogens was made in the French Pacific territory of Wallis and Futuna by the SPC and the Wallis and Futuna Service de l'agriculture.



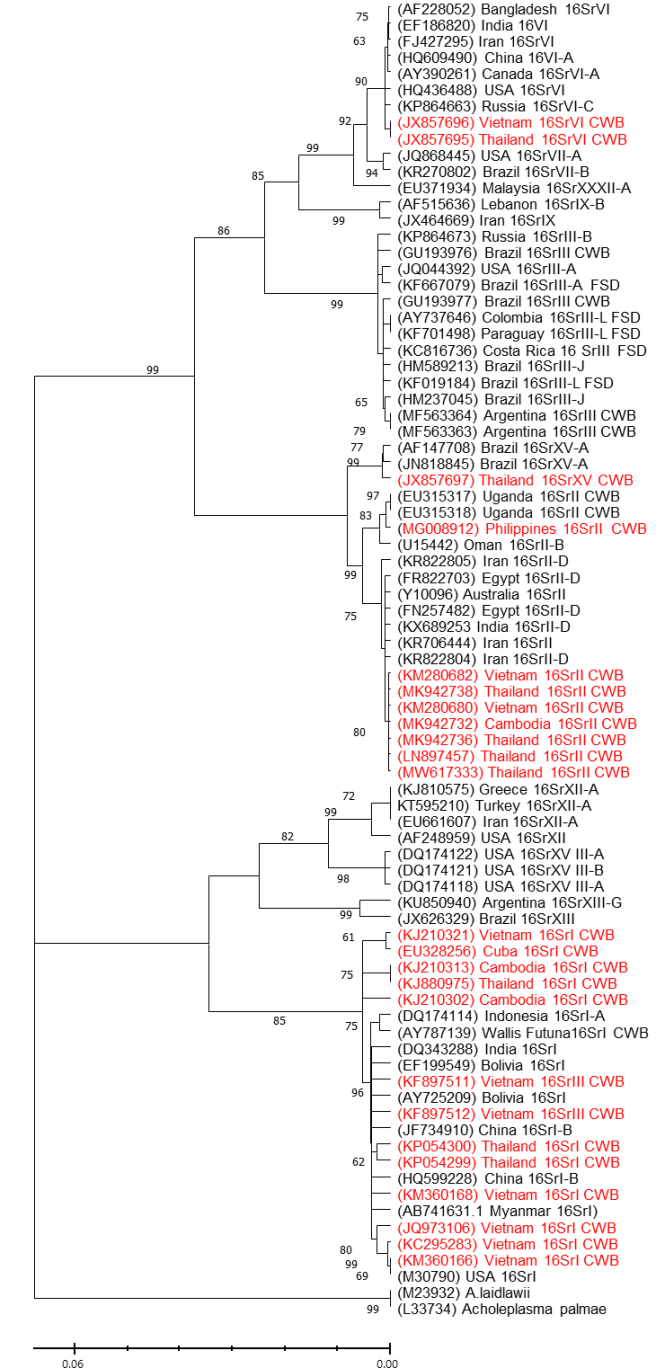
Symptoms of witches' broom (proliferation of axillary buds), yellowing and little leaf.

Cassava little leaf AY787139 99% to aster yellows strain CHRY (AY180950) 'Ca. Phytoplasma asteris' (16SrI)

Source: Personal communication with Richard Davis, Plant Pathologist - Northern Australia Quarantine Strategy (NAQS)

During more than 10 years…..

The CWB disease in SEA was observed during 2008-2009



CMD in SEA emerged after a ‘first wave’ of CWBD

- 2010: CWBD incidence ~80% in Yen Bai, Quang Ngai and Dong Nai (north, central and south Vietnam)
- 2012: CWBD incidences of 30-40% in Cambodia in the provinces of Kampong Cham, Kratie and Prey Veng.
- 2012: field surveys in Chachoengsao and Rayong in southern Thailand, report similar “high incidences” of CWBD.
- 2014: average incidence of CWBD in SEA 32% (highest in Cambodia, 46%)

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Pre-emptive disease management in SEA

Focus: CWBD, ***Bemesia tabaci***

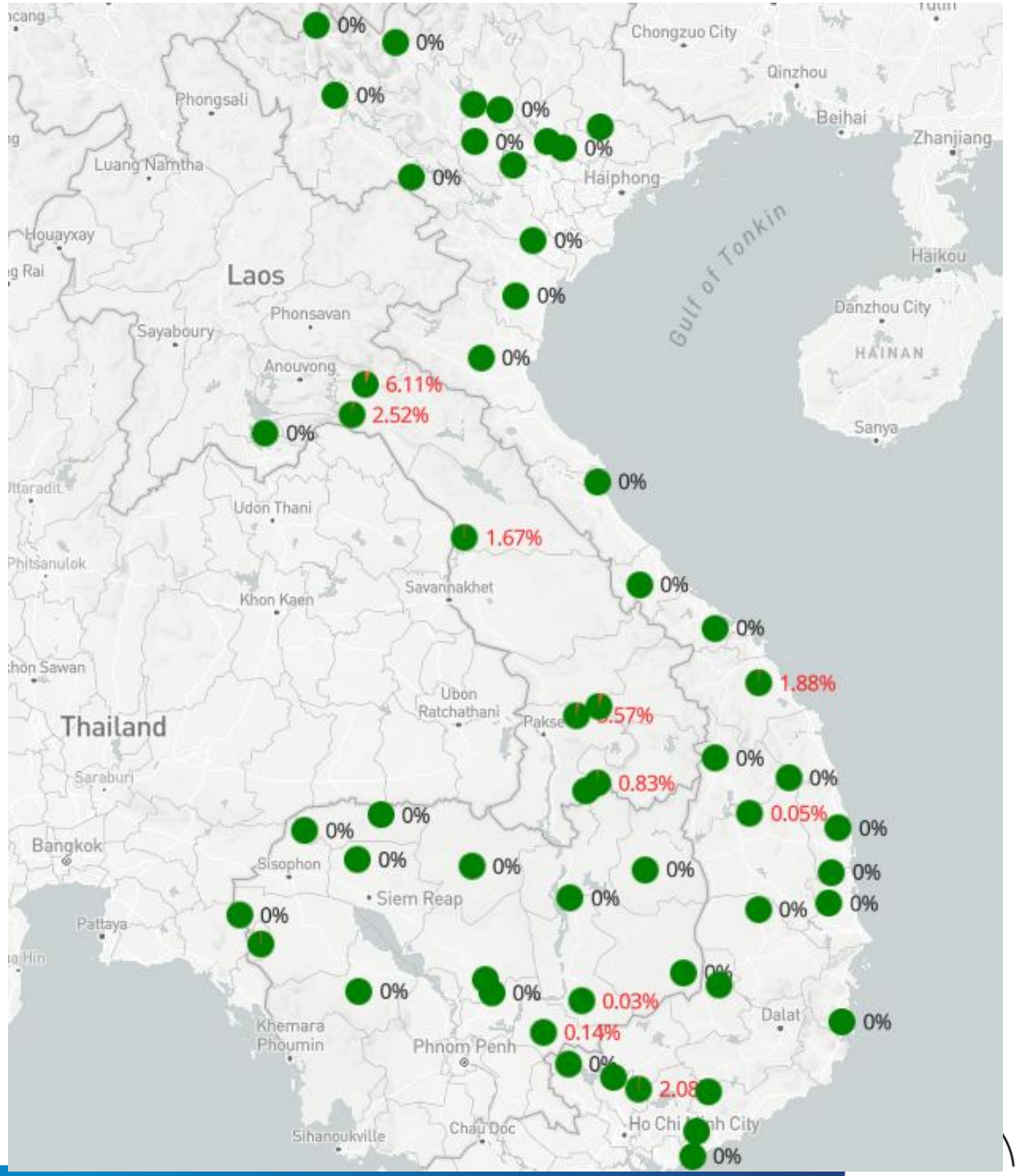
- ***What do we know?***
- ***What are we doing?***
- ***Next steps***

Cassava Retreat 2023, Warren Arinaitwe

CWBD Distribution

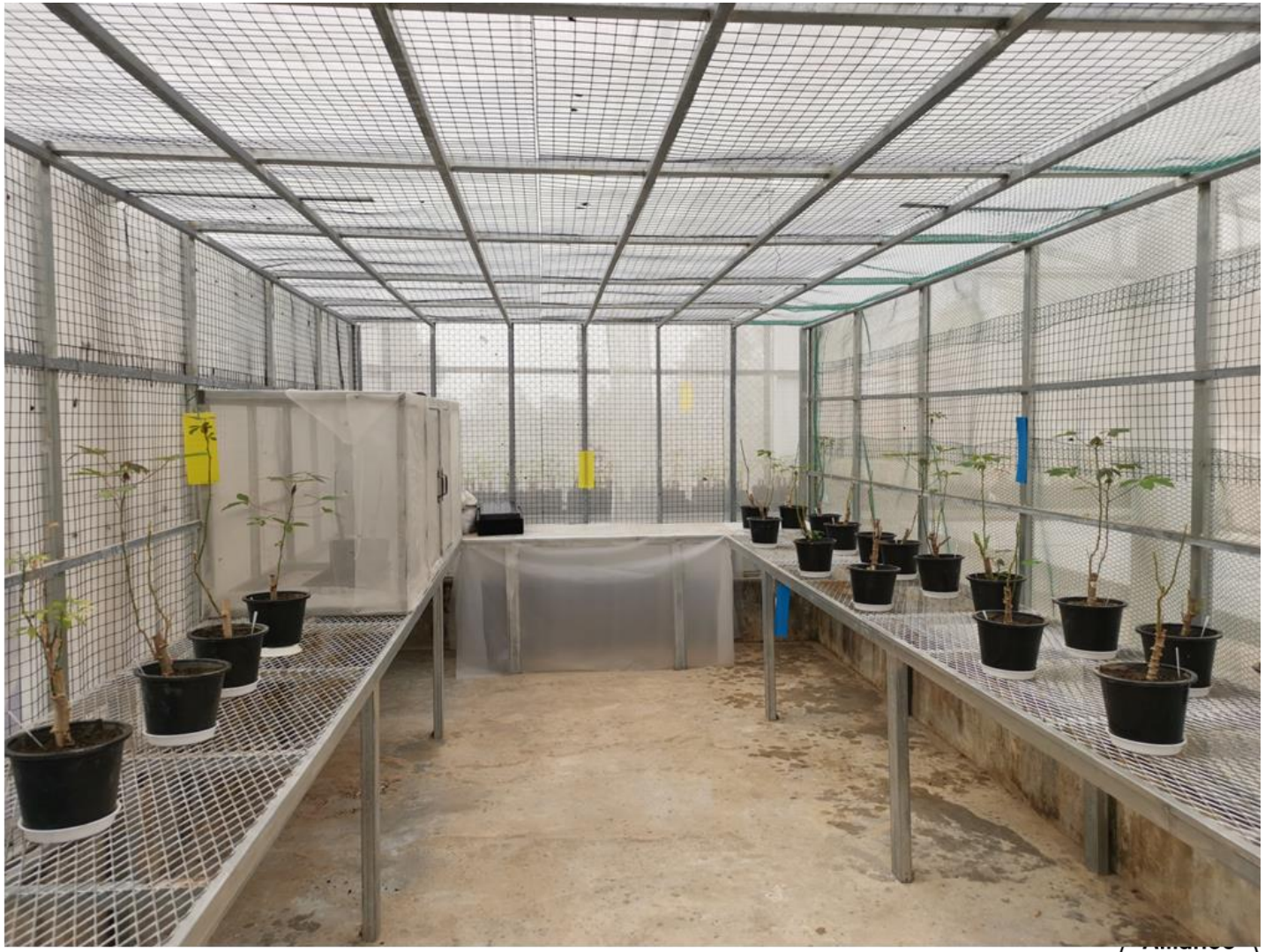
Field situation Dec 2022

Early-Mid season 2020-2021



Establishment of prerequisite infrastructure

New insect-proof screen house

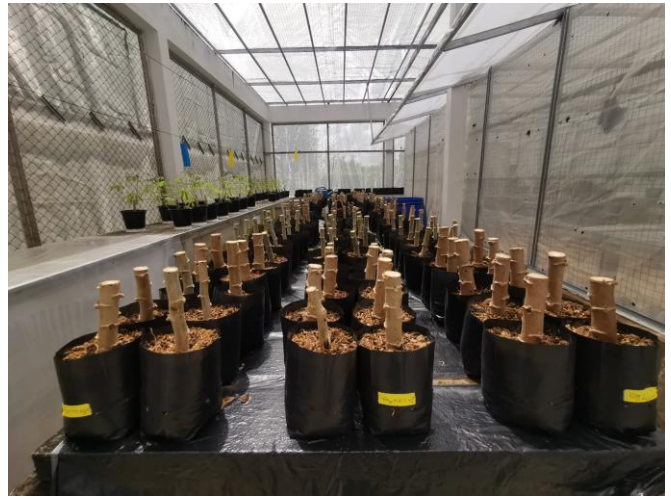


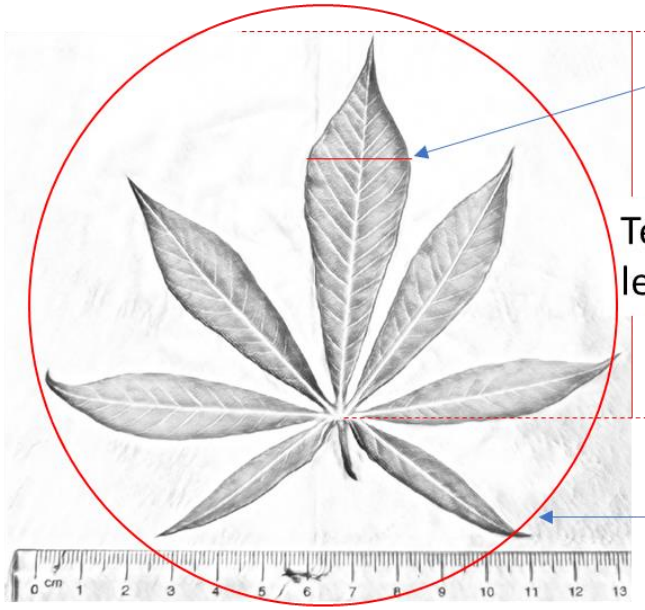
New molecular laboratory

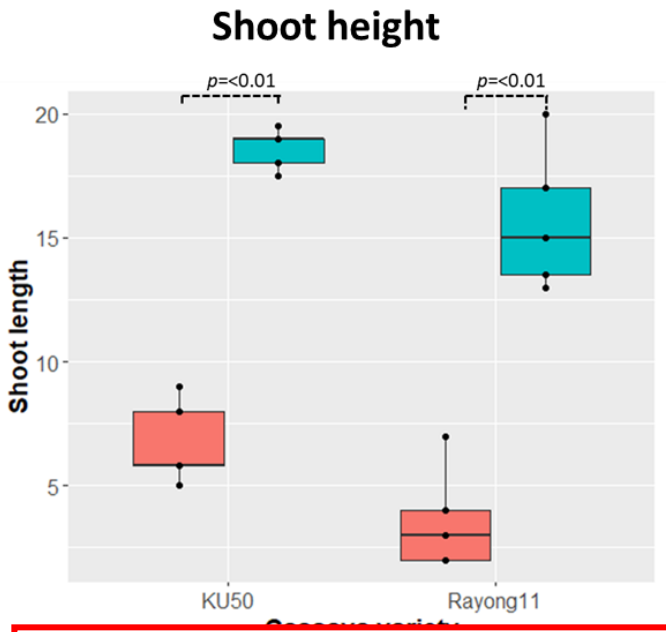
Old workspace **18 M²**

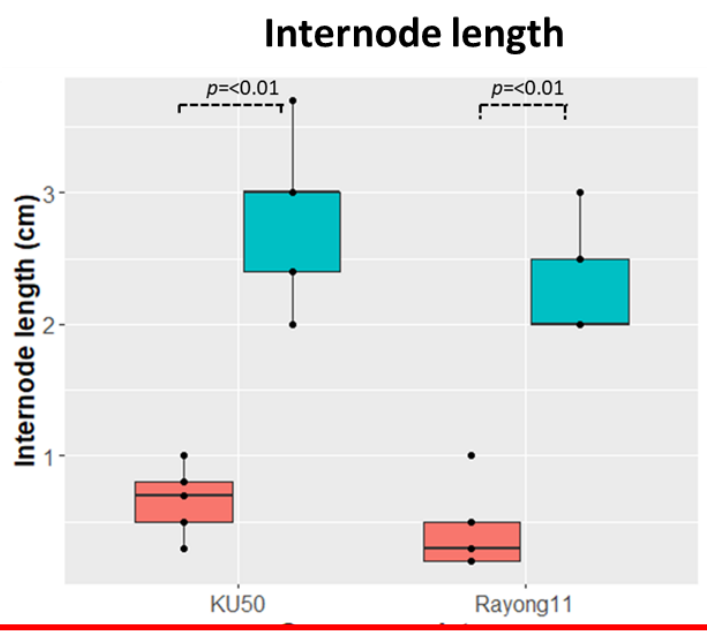
New workspace **94.5 M²**

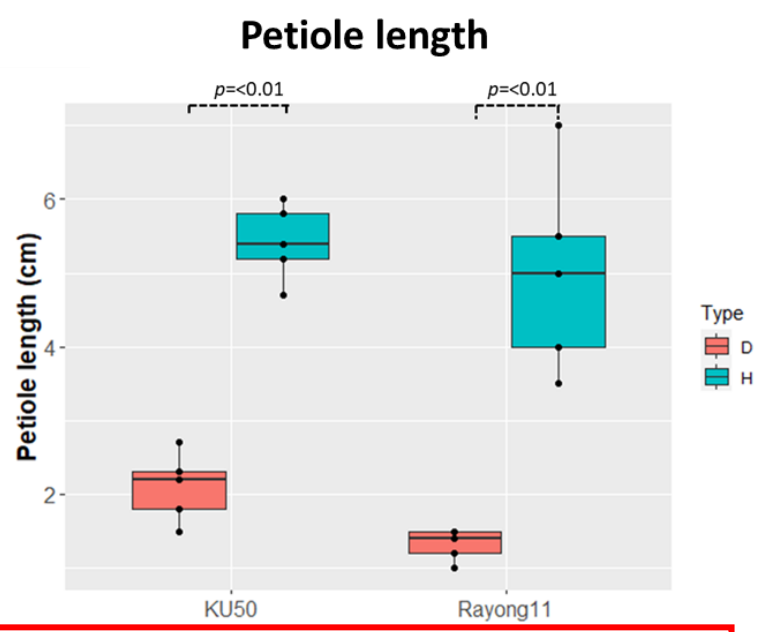
Understanding the impact of CWBD on phenotype



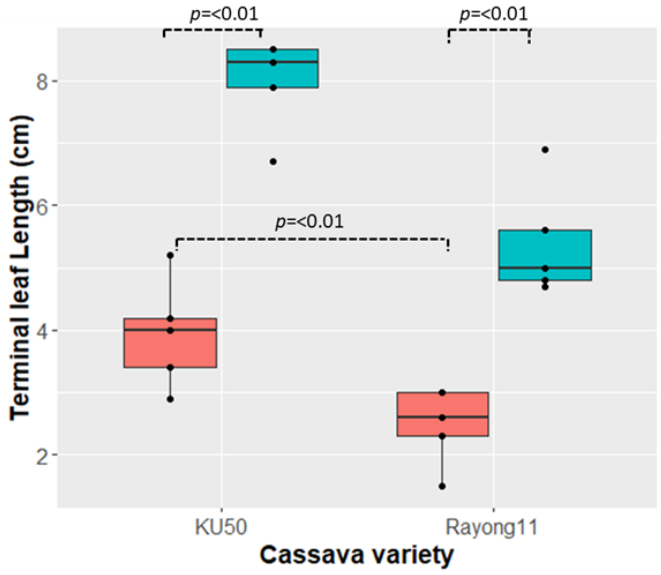


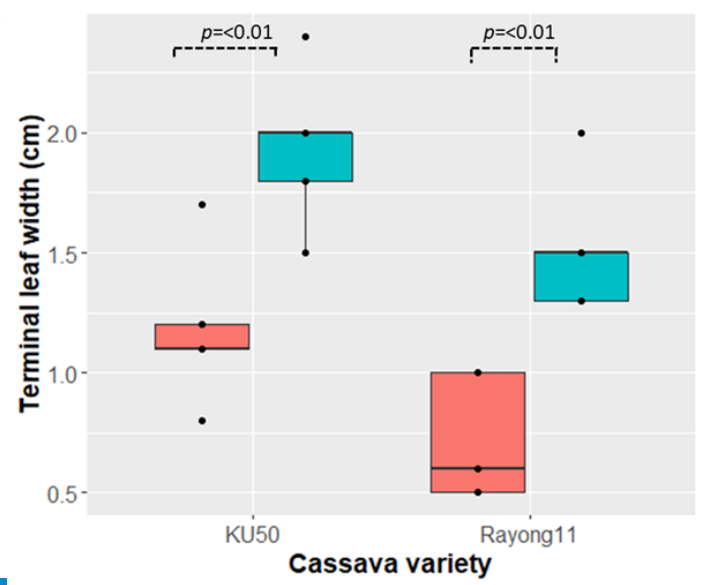


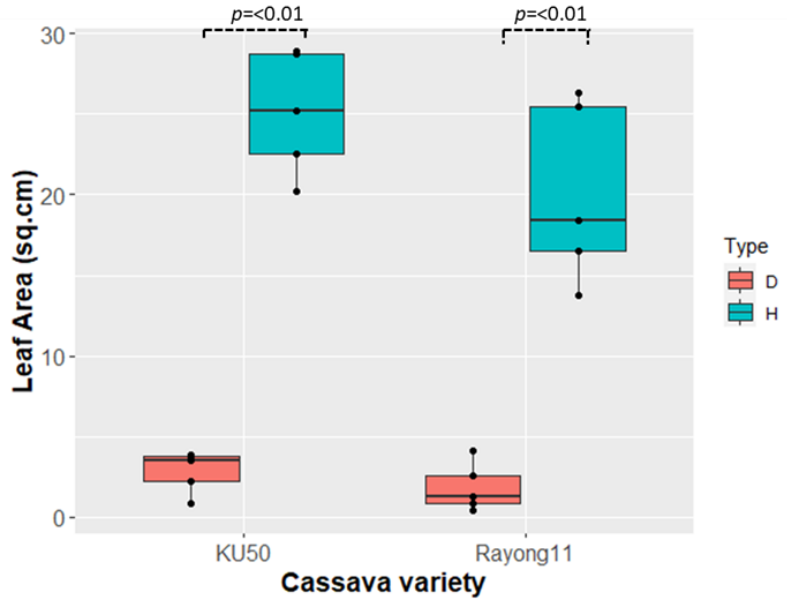




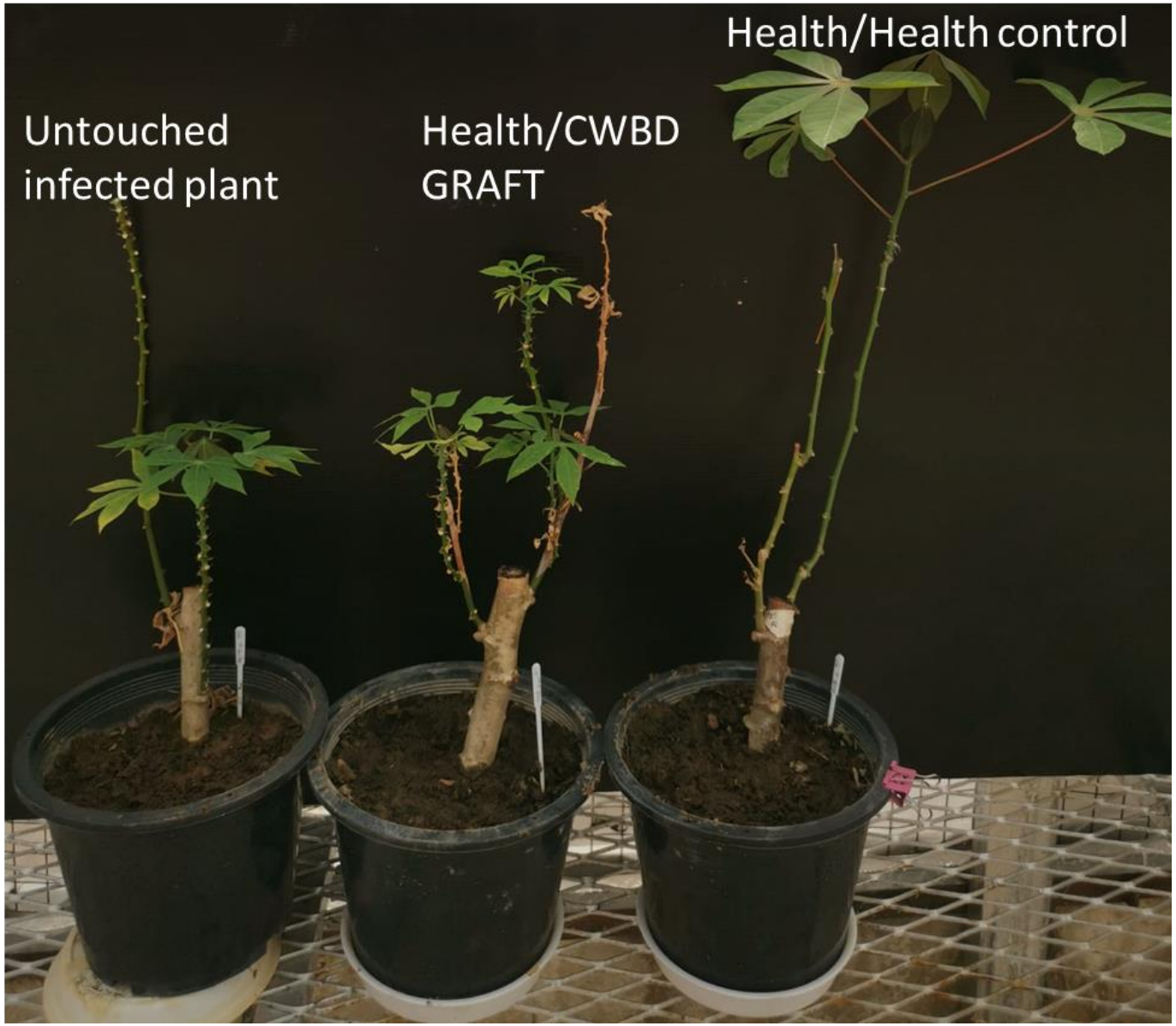
CWBD significantly limits the photosynthetic capacity of cassava







Can CWBD be transmitted by grafting?



Optimisation of CWBD DNA extraction, detection, symptom-pathogen association

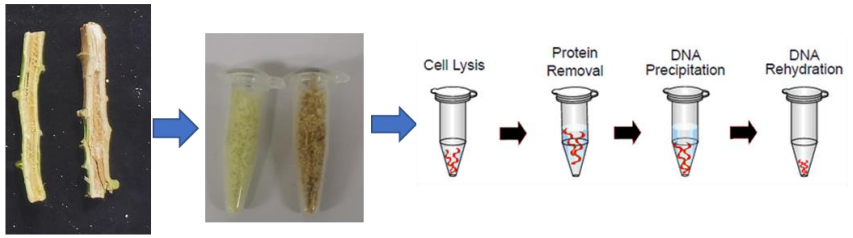
Target tissue?

X

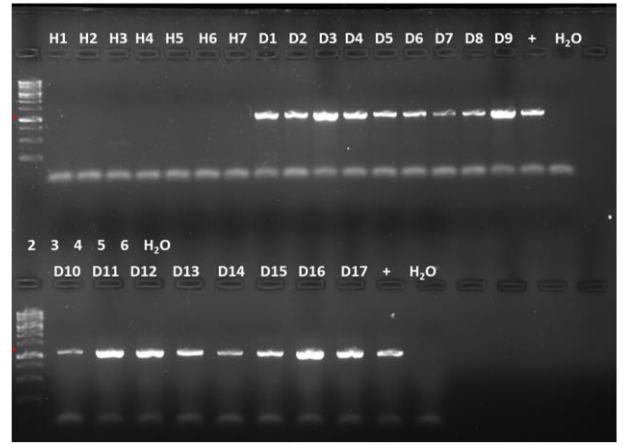
Stem… Yes!

Carefully chisel out spongy tissue (pith)

Carefully scrape off part of the vascular tissue



CTAB extraction



H= healthy; **D**= CWBD infected plants

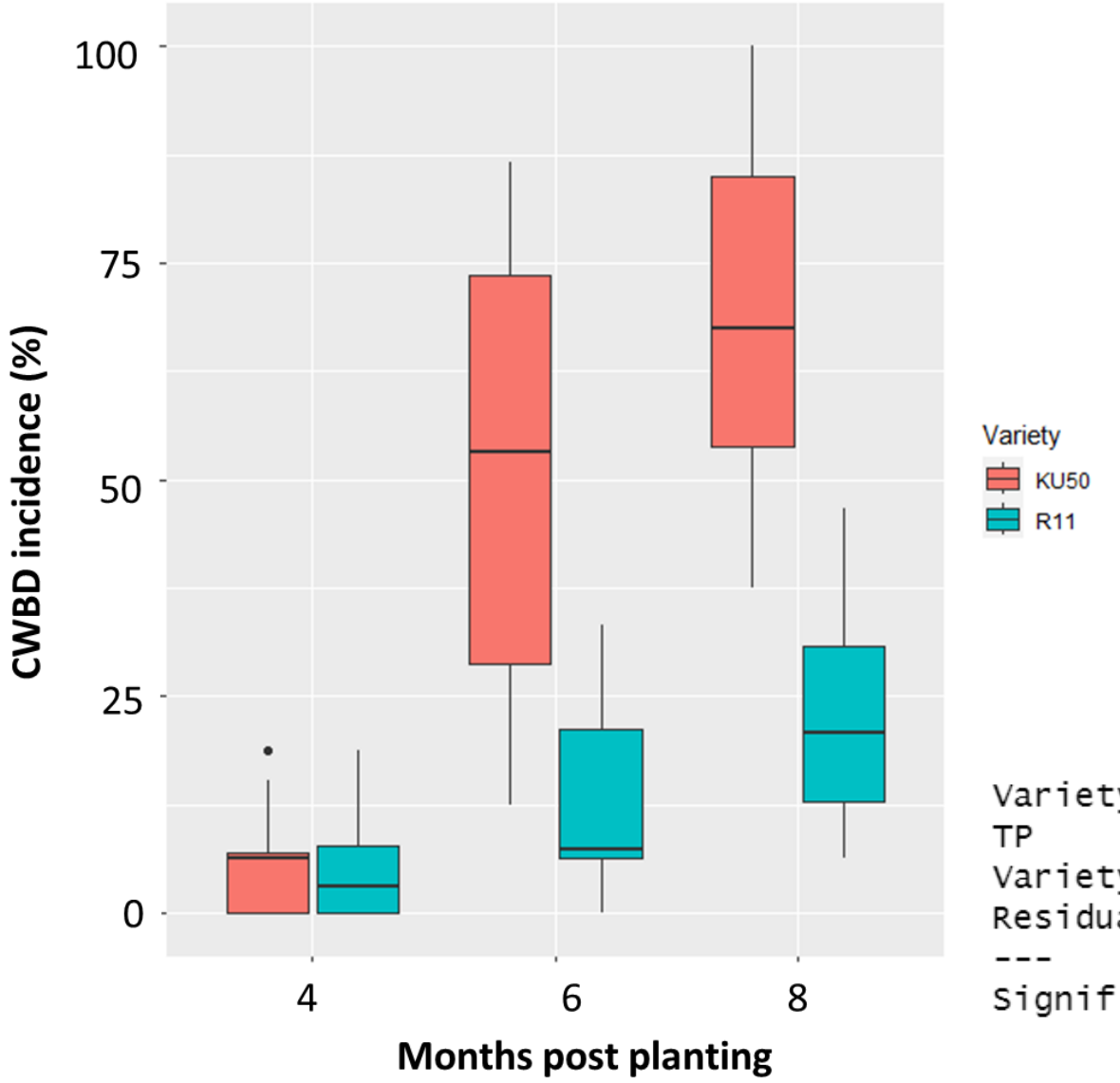
Take-home: Vascular stem tissue is a better target for the extraction of CWBD DNA

Summary of PCR results from samples collected from CWBD infected field in Vientiane

		Ceratobasidium detection	
Phenotype	**No of samples**	**PCR (-)**	**PCR (+)**
Asymptomatic	20	19	**1**
Classic CWB	31	0	**31**
Other symptoms	3	3	0
Total	**54**		

Field experiments
Warren, LaoThao, Rith and Imran

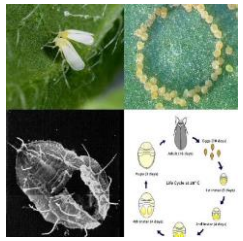
CWBD pressure changes with plant growth stage

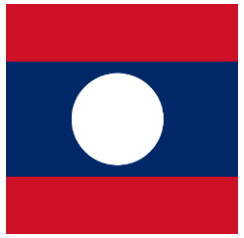


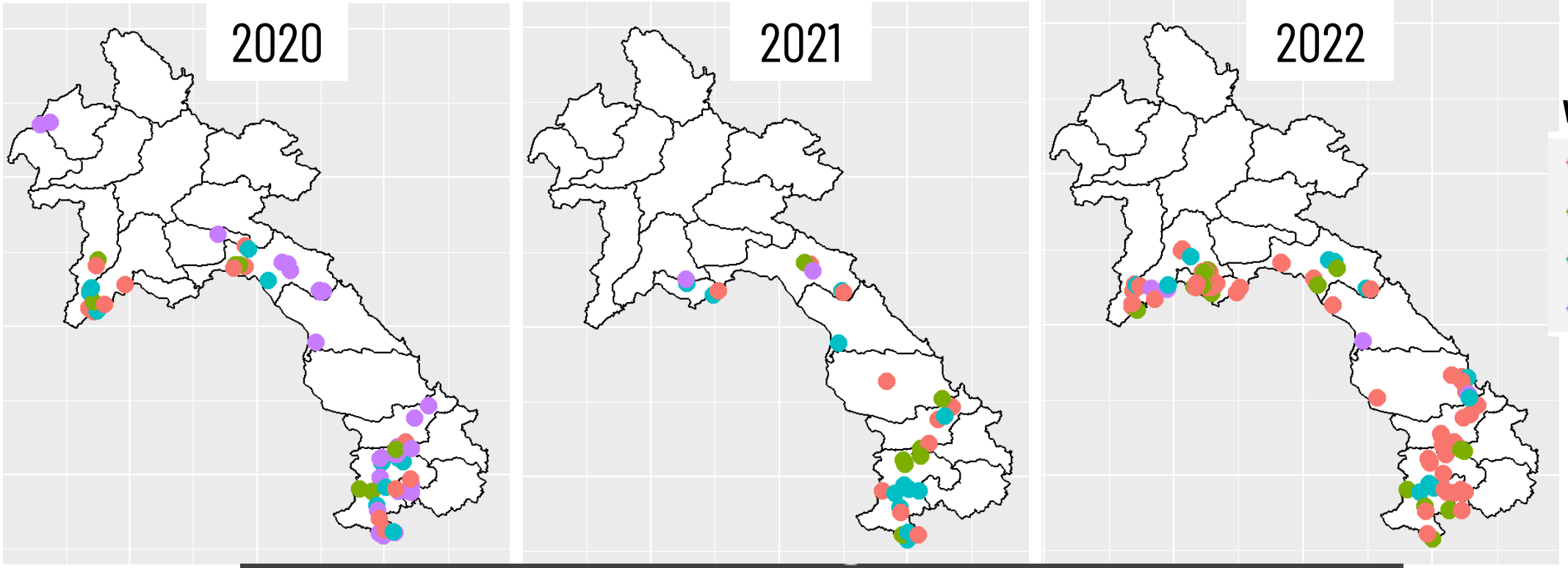
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Variety	1	29141	29141	133.06	< 2e-16	***
TP	2	40527	20264	92.53	< 2e-16	***
Variety:TP	2	14662	7331	33.47	1.41e-12	***
Residuals	138	30223	219			

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

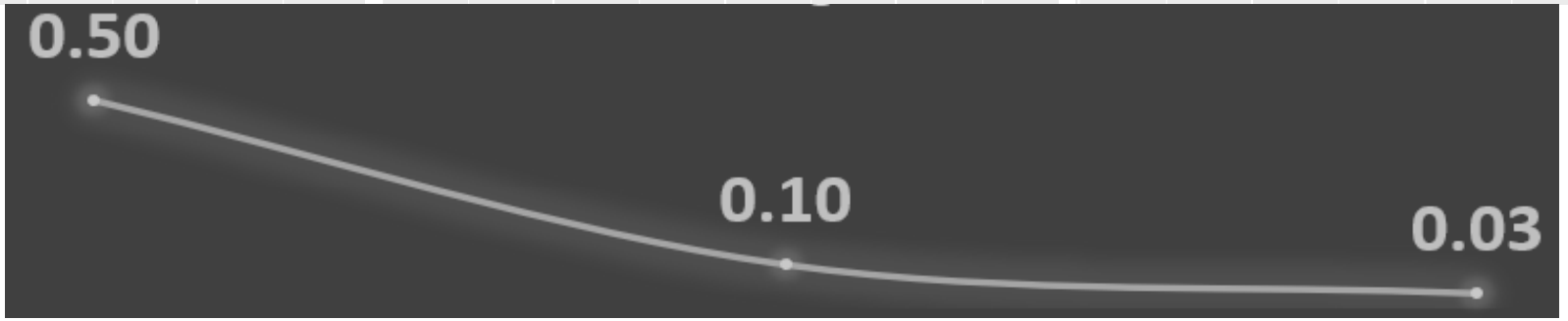
Whitefly surveillance







Mean abundance



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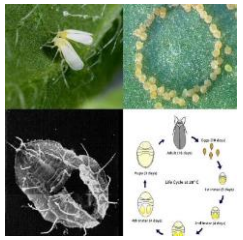
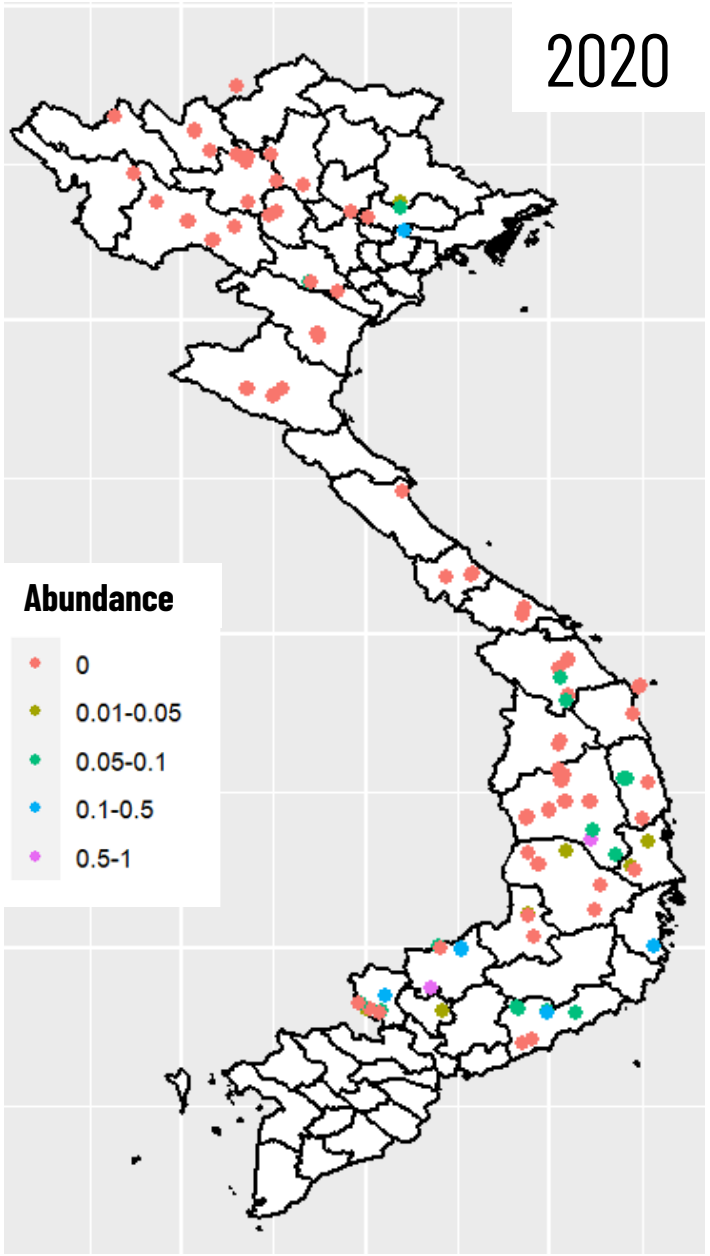
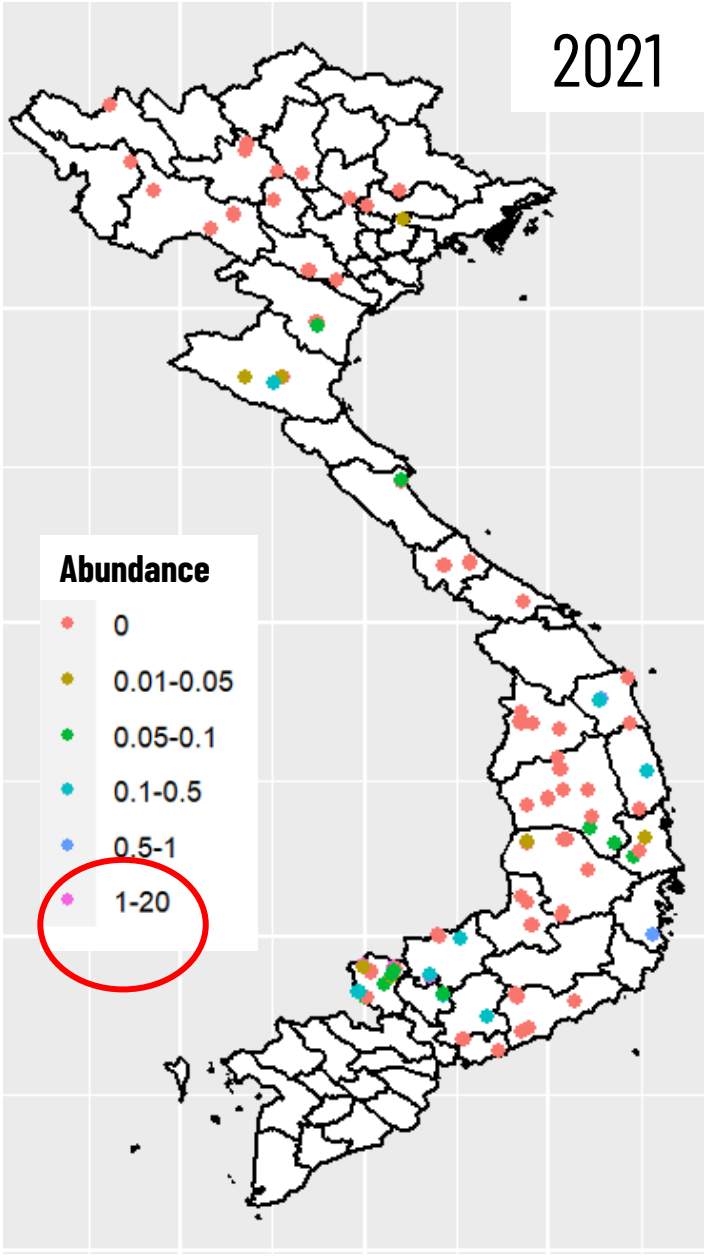


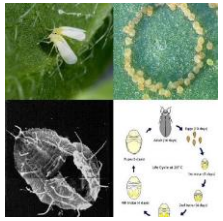
2020



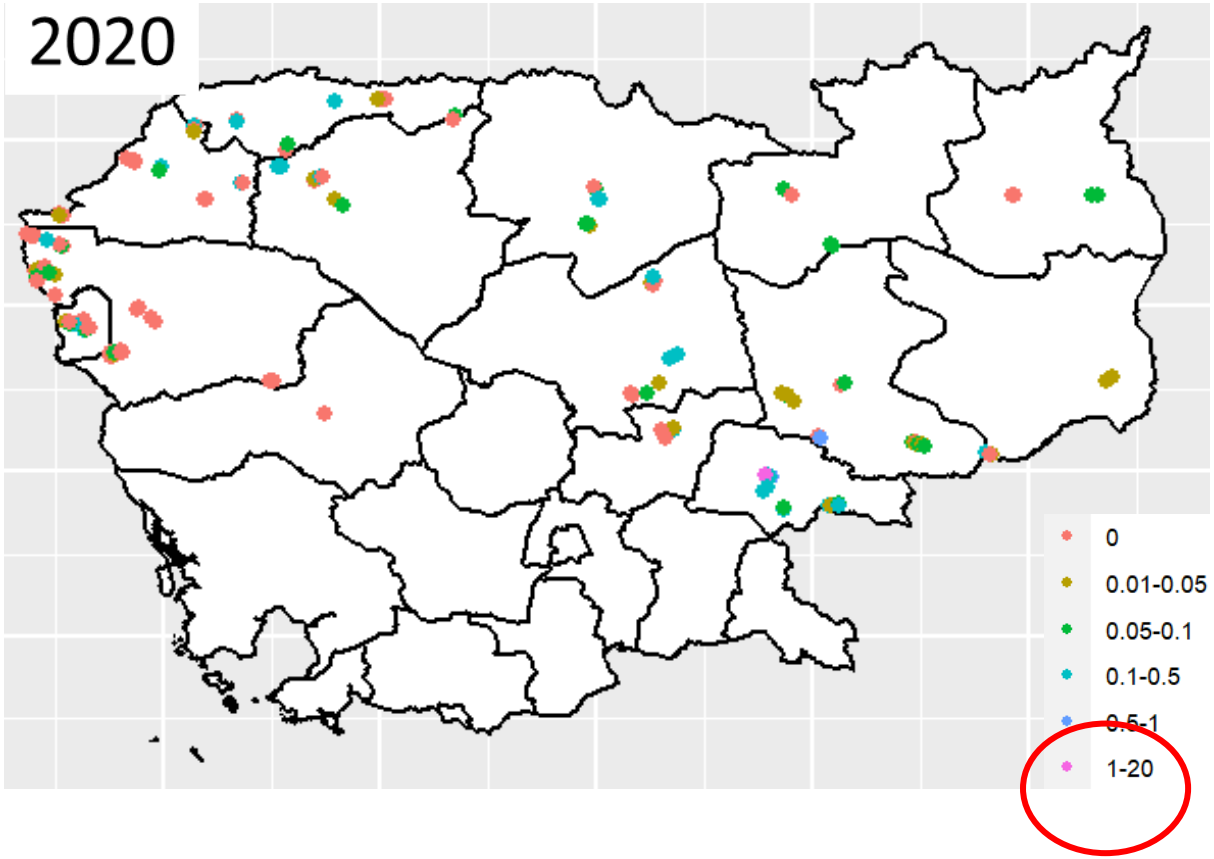
2021



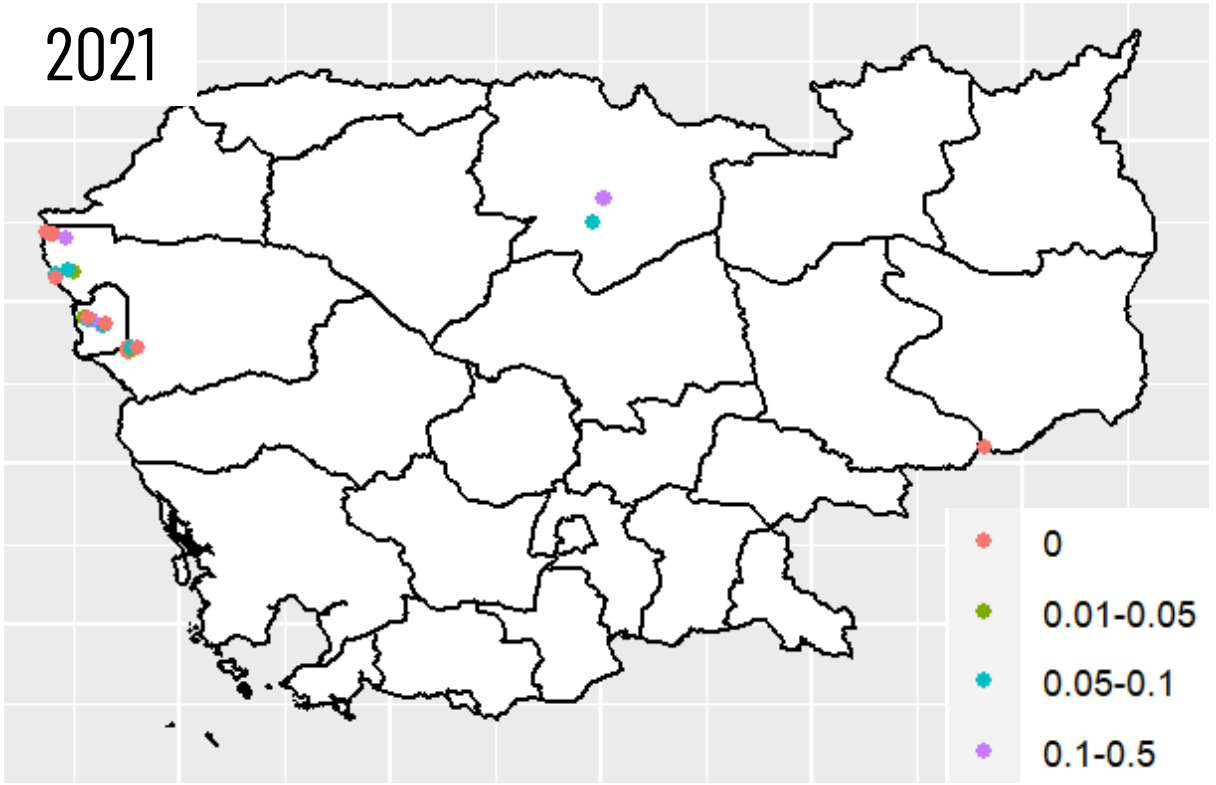
Whitefly population building up!



2020



2021



High-throughput sequencing (HTS) methods

Illumina MiSeq

Pacific Biosciences Sequel II

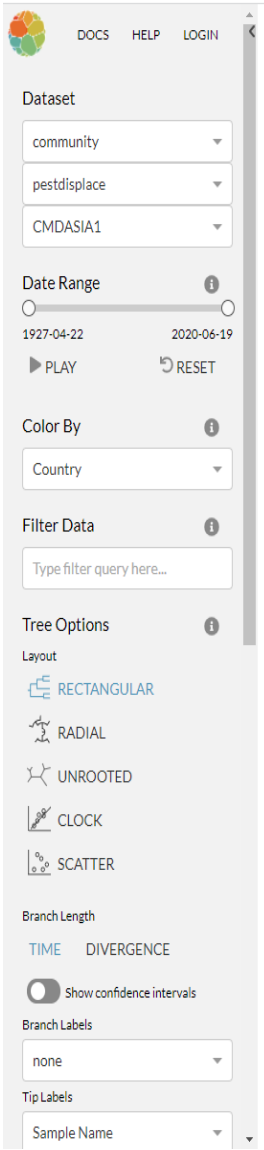
Oxford Nanopore PromethION

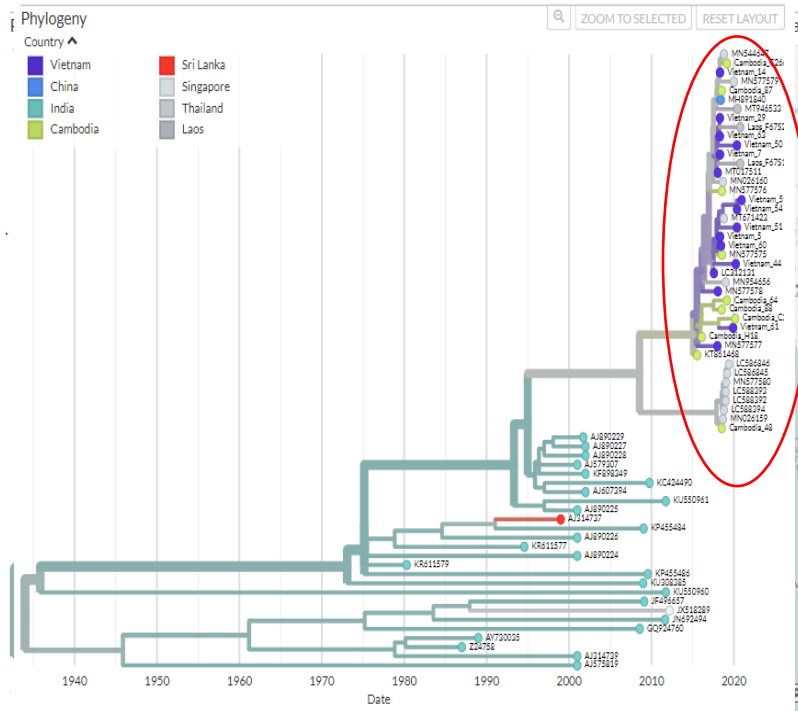
Illumina NovaSeq 6000

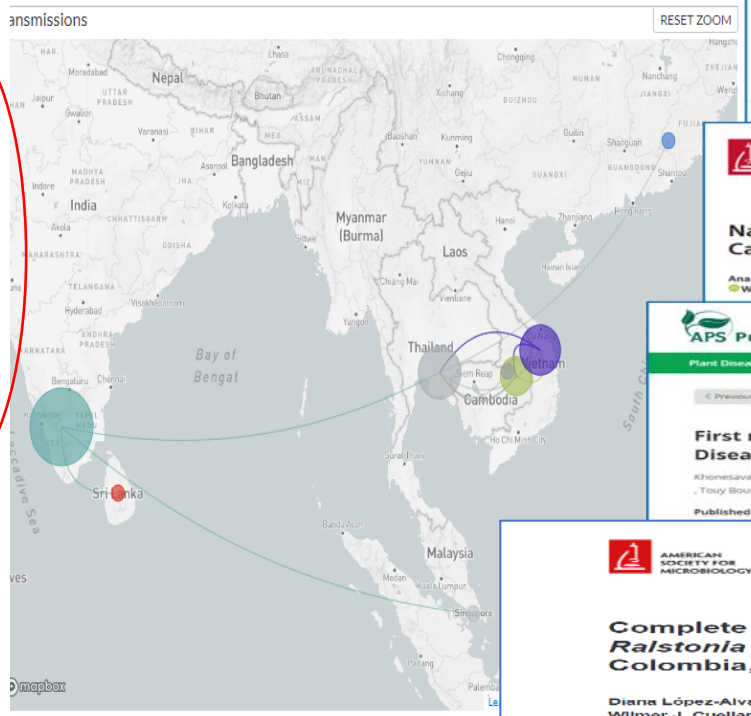
Pacific Biosciences RS II

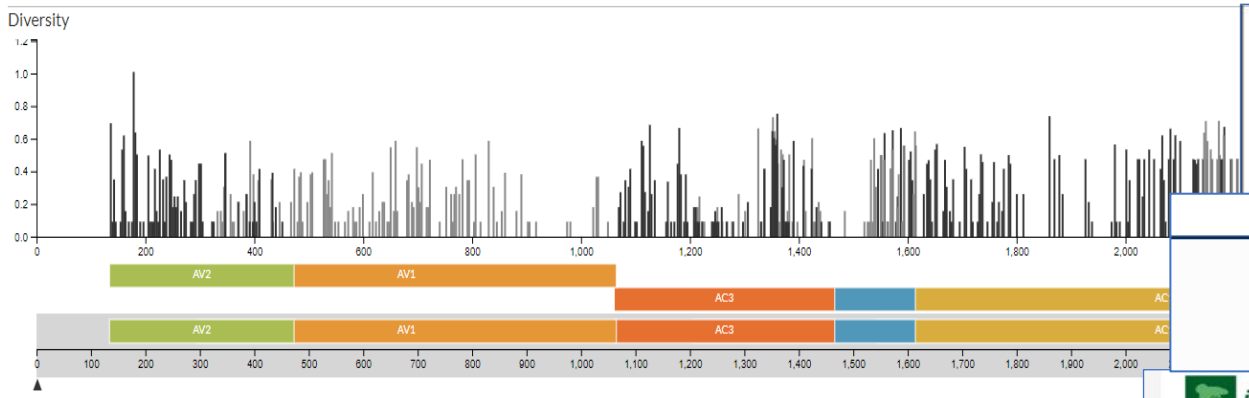
Oxford Nanopore MinION

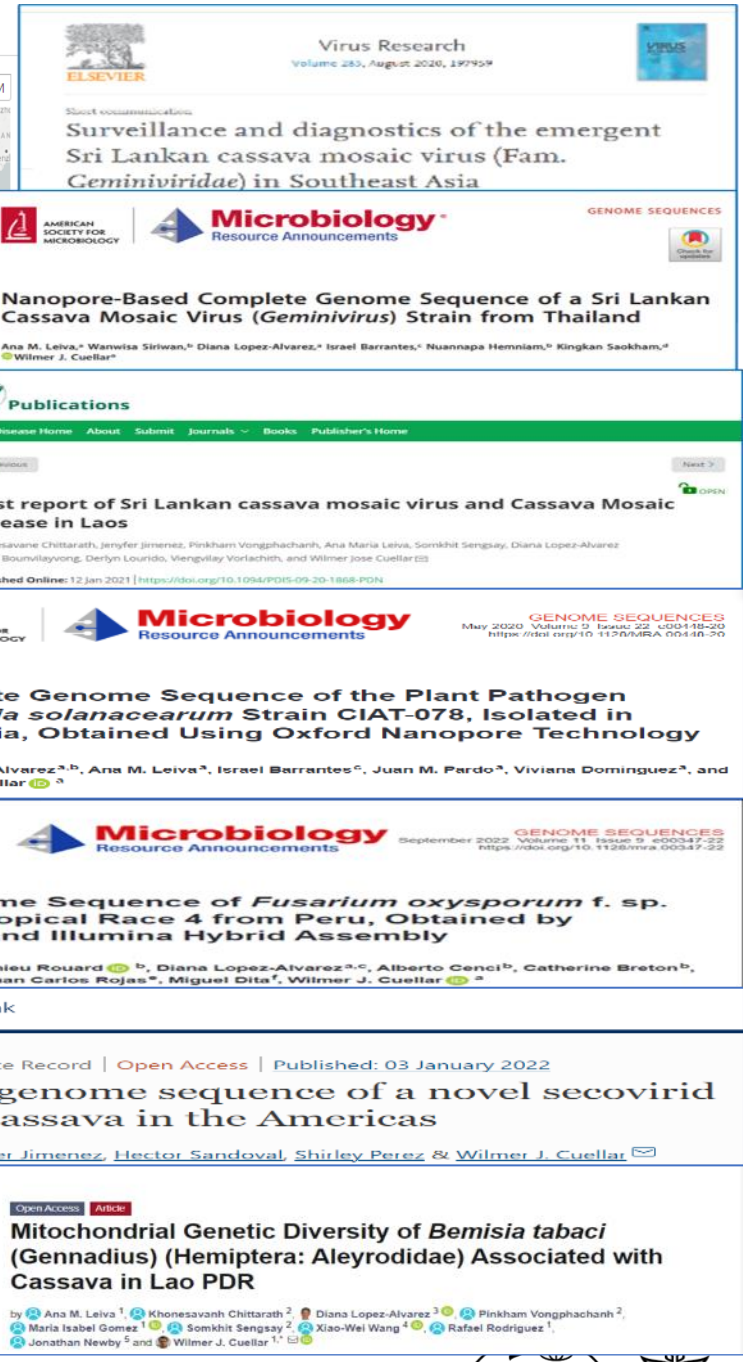
Oxford Nanopore SmidgION











https://nextstrain.org/community/pestdisplace/CMDASIA2

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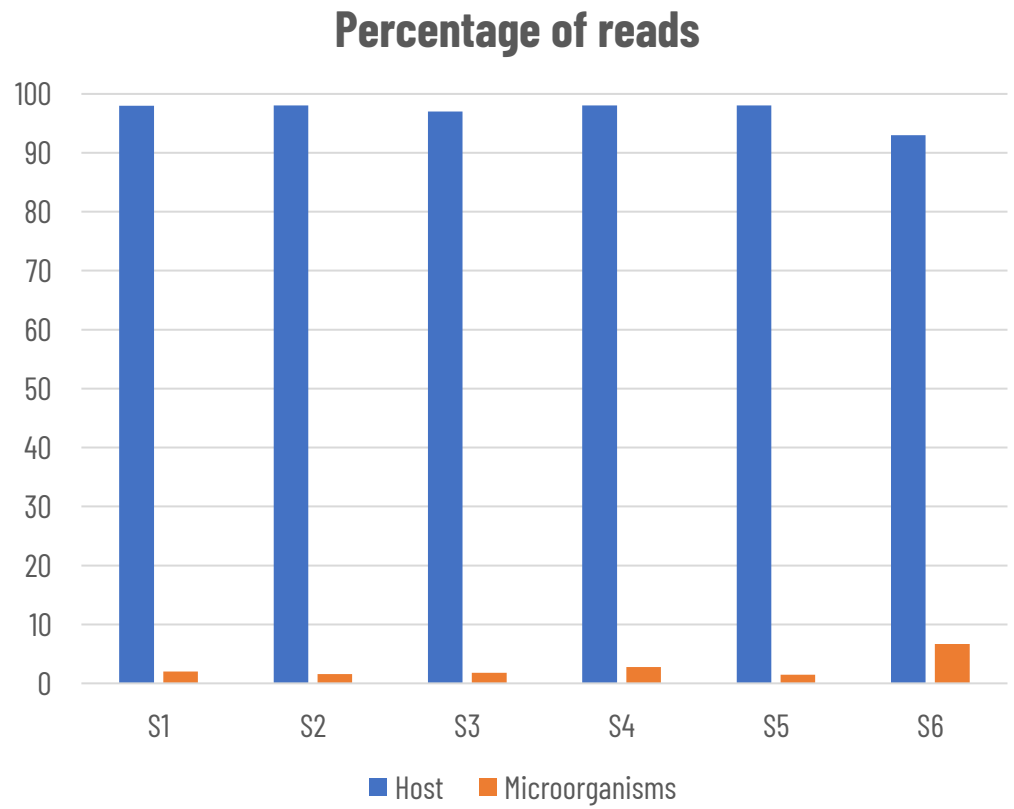
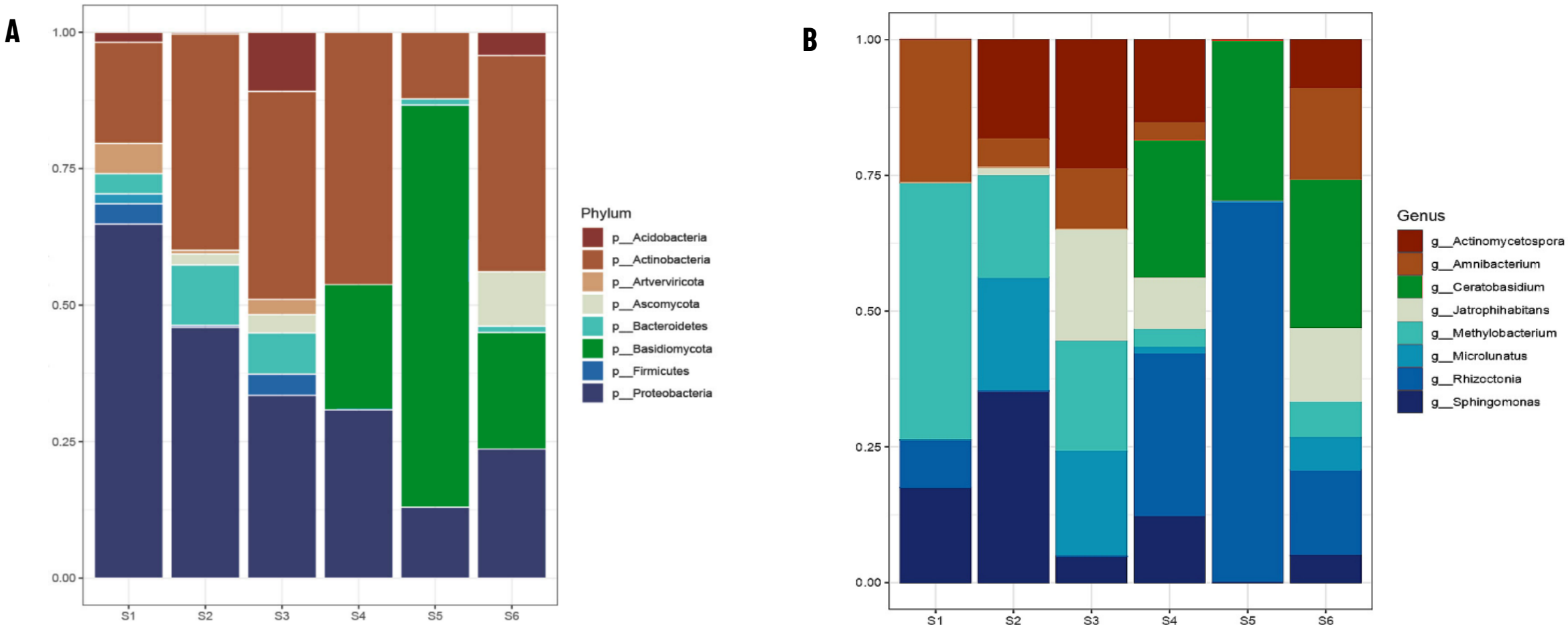
Comparative Metagenomic in CWB



Sample	Stem
S1	Asymptomatic
S2	Asymptomatic
S3	Asymptomatic
S4	Symptomatic
S5	Symptomatic
S6	Symptomatic

Experiment using whole genome sequencing (Hiseq illumine). comparison of asymptomatic with symptomatic plants.
Produce more than 2 billion of reads per sample.

Barplot: Relative frequency classified



Barplots showing the relative abundance of different taxons in six samples from stem cassava. **S1-S3**: Asymptomatic stem. **S4-S6**: Symptomatic stem with witch's broom disease. Barplot A shows the relative abundance of different genus. Barplot B shows the relative abundance of different phylum. Notice that only in Symtomatic samples we find Basidiomycota. **Y:** Abundance classified **X:** Samples.

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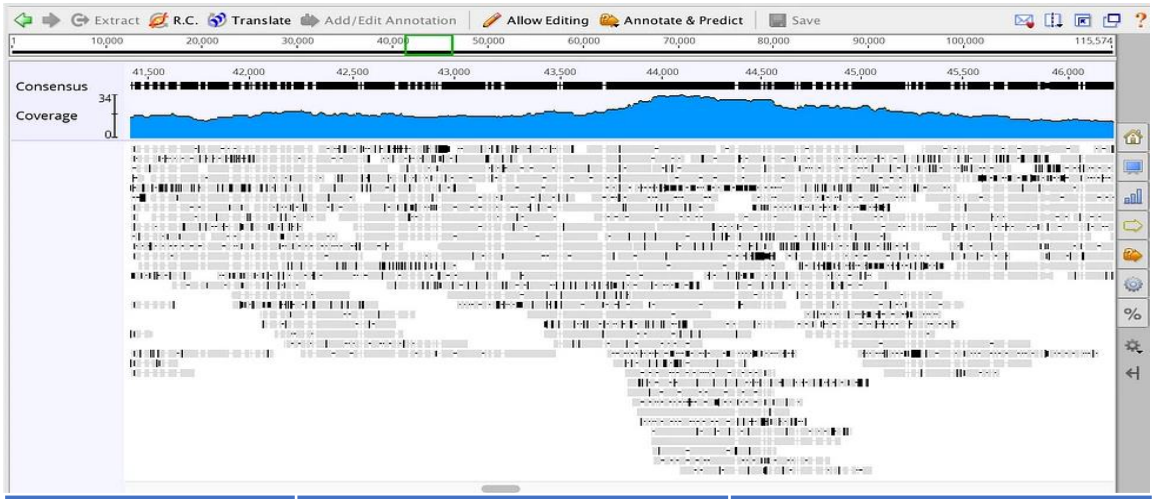
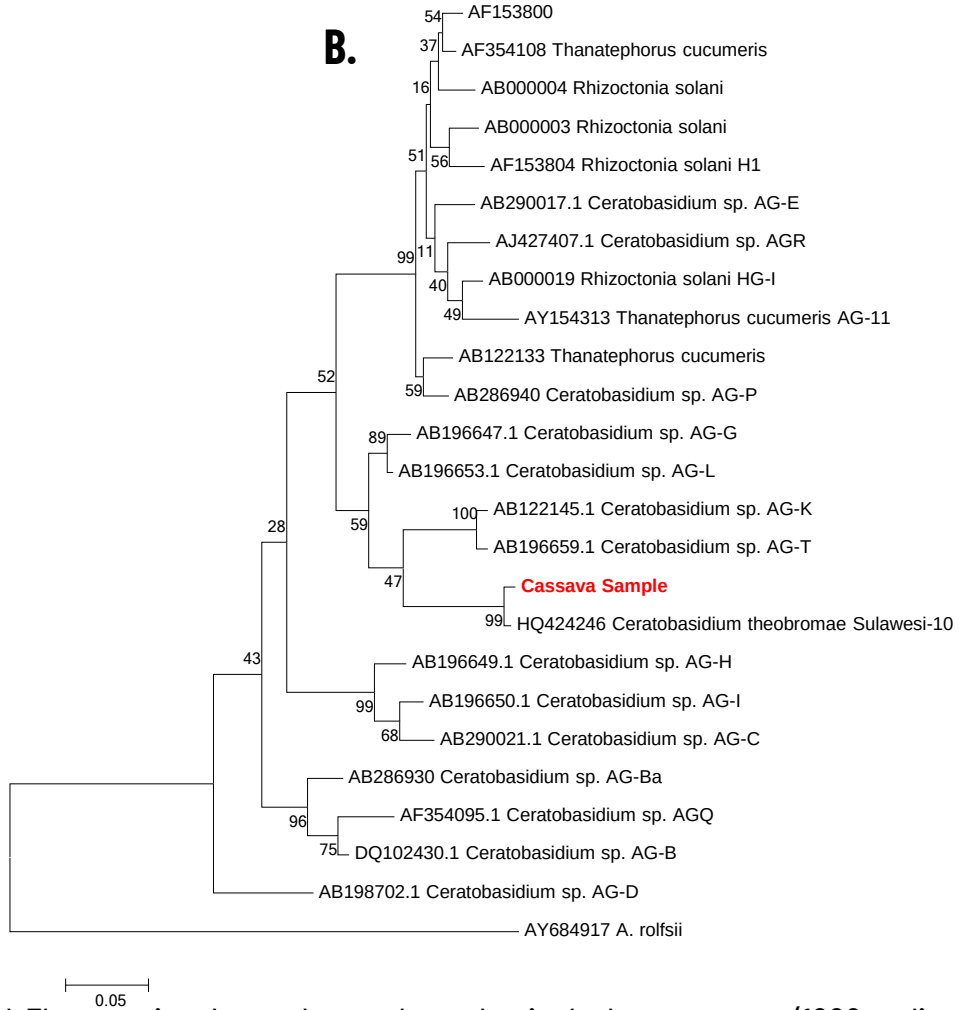
Designer of primers to detection and using ITS region to classify the fungal

A.



3 set of Primers	Forward	Reverse
Cerato1	GGTTGATTGCCCAGGGACTGC	CTTGTACTGCGAGCTCTCACG
Cerato2	**GGATGAGTTTAATCGCTCTAAC**	**GCGCTCTGGTGTTTCAAGTTTG**
Cerato3	CGTCGTTGGGCTAGTTATTGC	CGTATCCCCTAGCCATGGAAC

B.



A. Primer design using geneious program **B.** Phylogenetic tree using the Neighbor-Joining method. The associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method. All positions containing gaps and missing data were eliminated. There were a total of 556 positions in the final dataset. Evolutionary analyses were conducted in **MEGA.**

Sequencing of Ceratobasidium from cassava

All statistics are based on contigs of size >= 3000 bp, unless otherwise noted (e.g., "# contigs (>= 0 bp)" and "Total length (>= 0 bp)" include all contigs).

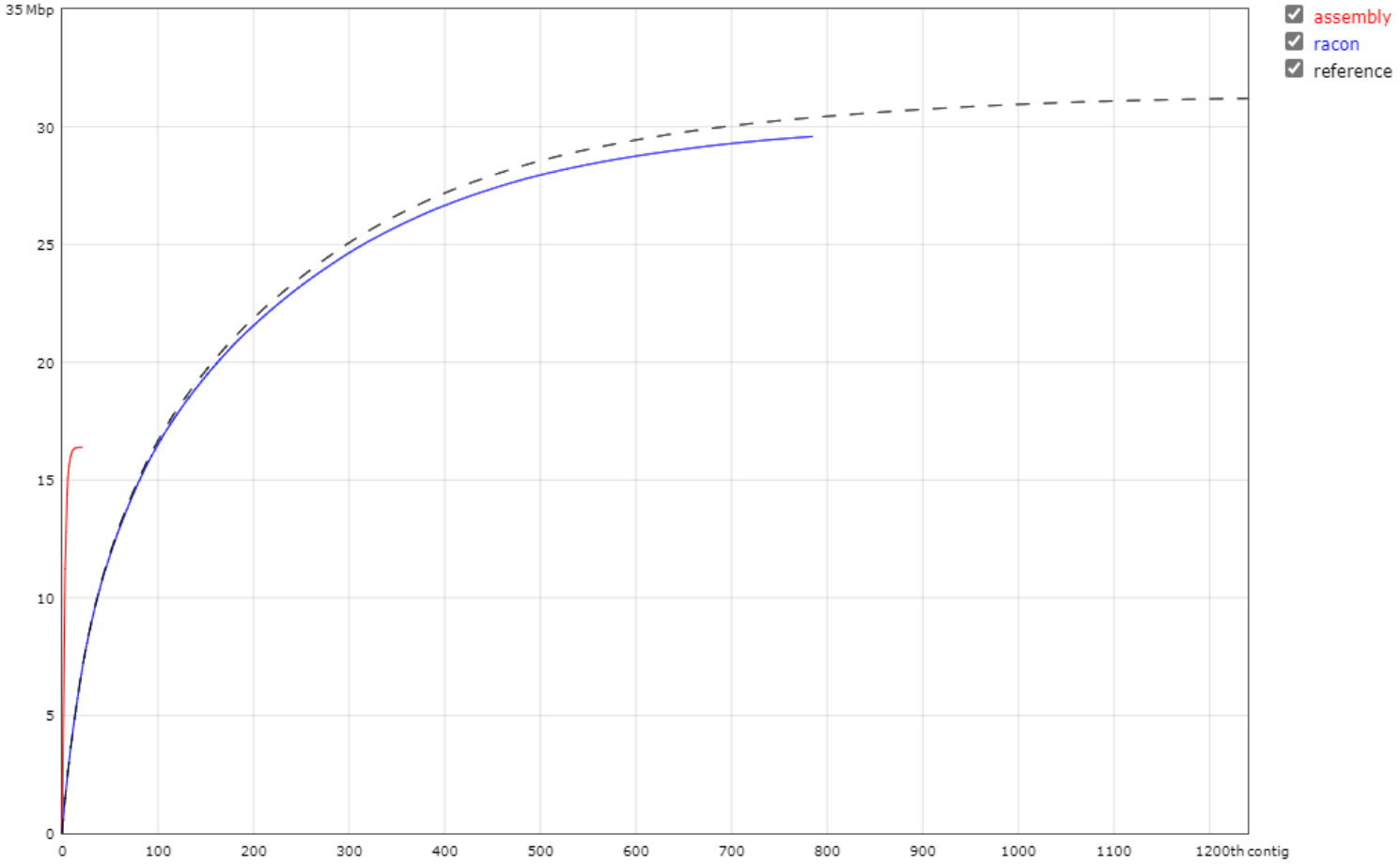
Aligned to "GCA_009078325.1_ASM907832v1_genomic" | 31 201 167 bp | 1240 fragments | 49.22 % G+C

Worst Median Best — Show heatmap

Genome statistics	assembly	racon
Genome fraction (%)	0.045	71.313
Duplication ratio	1.213	1
Largest alignment	4710	522 419
Total aligned length	16 577	22 237 052
NGA50	-	38 616
LGA50	-	139
Misassemblies		
# misassemblies	0	1
Misassembled contigs length	0	9026
Mismatches		
# mismatches per 100 kbp	5621.11	4071.83
# indels per 100 kbp	100.12	149.83
# N's per 100 kbp	0	0
Statistics without reference		
# contigs	21	785
Largest contig	3 963 473	587 751
Total length	16 395 283	29 588 205
Total length (>= 1000 bp)	16 397 213	30 076 093
Total length (>= 10000 bp)	16 365 530	27 921 243
Total length (>= 50000 bp)	16 270 605	19 303 542

Extended report

Plots: Cumulative length | Nx | NAx | NGx | NGAx | Misassemblies | GC content



Contigs are ordered from largest (contig #1) to smallest.

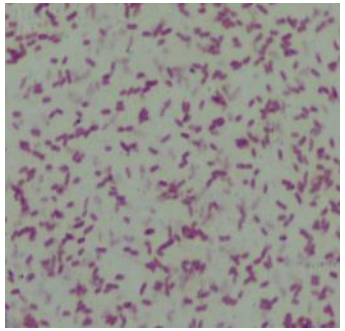
Cassava Bacterial Blight (CBB)

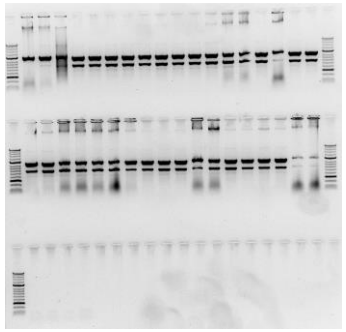
Juan M. Pardo, Rafael Rodriguez

Bacterial Blight disease progression, using machine-learning image analysis

1. Isolate *Xantohomonas phaseoli* pv. *manihotis* (Xpm)

2. Confirm the Xpm identity (Bernal-Galeano et al., 2018)





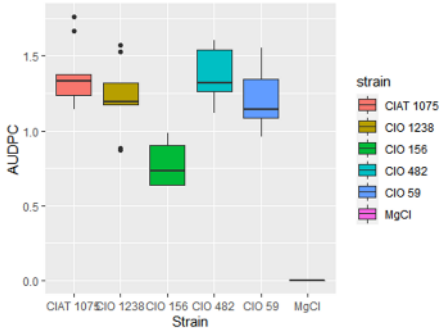
3. Prepare the inoculum: 3 days at 25˚C, dilution 0.01 OD600

4. Choose the three leaf from 4–5-week-old cassava plants

5. Inoculation through infiltration, 3 technical replicates per strain onto the leaf lobe

6. Three infiltration per lobe, central three lobes used, each lobe is a different strain.

7. Measure the spot area advance: phenotyper –Machine learning



Daily AUDPC advance

$$\mathrm{AUDPC} = \Sigma_i.[(D_i + D_{i-1}) * (t_i - t_{i-1})]/2,$$

Relative humidity=80%
Day/night temperature=28/19˚C

Per plant one leaf inoculated:
Lobe 1: CIO 81
Lobe 2: CIO 59
Lobe 3: Control (Water)
Per strain 3 plants

Measure in following days: 0, 4, 6, 9,12, Analyze data on R

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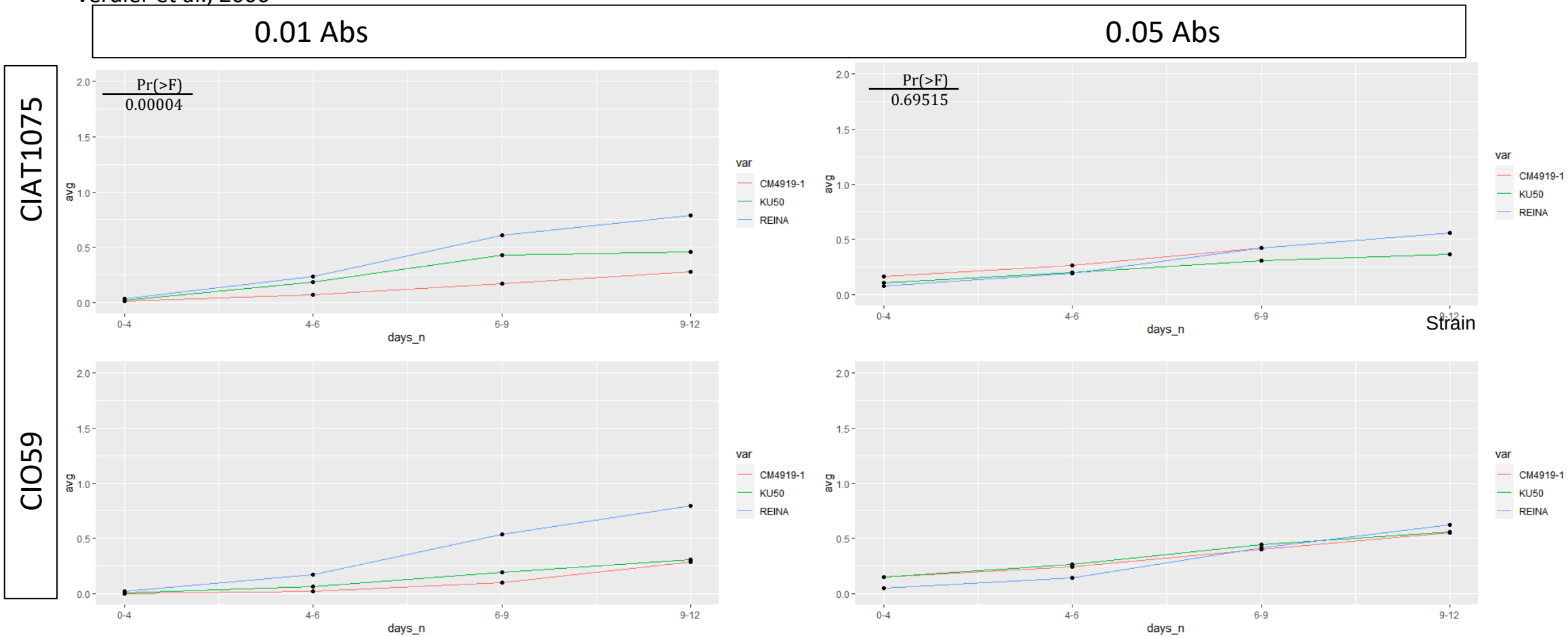
Data analysis

Susceptibility test

- Experimental design: Split plots . (genotypes, strains, three replicates, 9 observations per strain X Genotype). Negative control MgCl.

Strain	**Edaphoclimatic zones**
CIO 59	1-Subhumid tropics*
CIAT 1075	5-High altitude

*Verdier et al., 2000



Perspectives

Cassava root rot

https://www.flickr.com/photos/iita-media-library/7656614828/in/photostream/lightbox/

Detached storage roots of cassava **A.** Negative control **B**. Inoculated root with *Phythophtora* spp. The arrows point to the inoculation site and disease development region.

Source: Medeiros-Lima et al., 2018
https://doi.org/10.1016/j.pmpp.2018.09.001

Ten days after inoculation, the total area colonized by the pathogen on the root pulp could be evaluated by digital image analysis

Outline

Why is this topic important;

what are the key research findings;

- Whitefly maps, low incidence, Asia II 1; CWBD incidences, no phytoplasma detection (Warren)
- Ampelloviruses global distribution, Cassava common mosaic virus, Secovirids (Jenyfer)
- Diagnostics for circular DNA (cassava geminiviruses)
- Validate 'super' technologies (field sequencing, lamp, smartphones for disease dx)-prevenir gasto

what are already out the outcome level and ready for scaling;

- Circular DNA detection
- Microbiome analysis
- Cassava pathobiome detection
- PestDisPlace
-

what are the emerging questions and priorities.

- Accelerate stakes/genotype availability
- Models for resistance identification
- Endemic versus introduced pathogens and transcontinental movement of seeds
- Identification of resistance-breaking strains
- Vector identification – management of disease transmission
- Yield impact (experimental plots) for risk analysis

What next?

- Regional-wide symptom-pathogen association studies
- Extensive transmission studies
- Intercellular pathogen localisation studies
- ..... management options

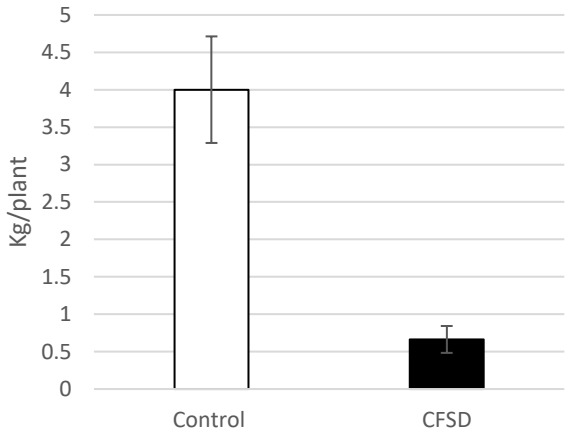
Cassava Frogskin Disease (CFSD)

Jenyfer Jimenez

Transmission

Injerto

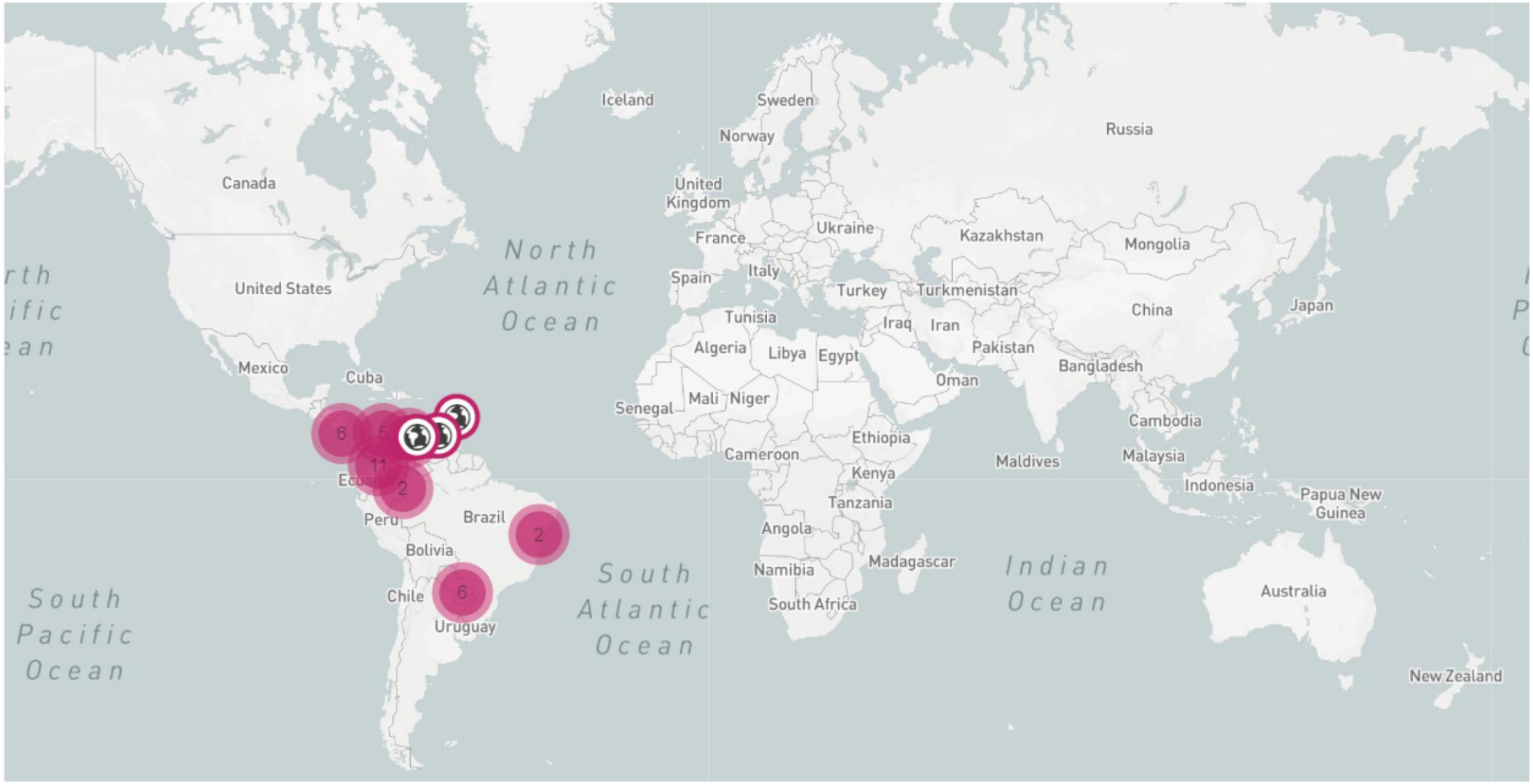
> 70%



Vector aéreo

Treatment	Seed type [a]	Average Severity of CFSD Root Symptoms (% Infected Plants)		
		1st Crop Cycle (2004–2005) [b]	2nd Crop Cycle (2005–2006) [b]	3rd Crop Cycle (2006–2007) [b]
Open field, no fumigation	CFSD	3.78 (77.3%)	3.72 (75.2%)	3.90 (79.3%)
Open field, no fumigation	Disease-free	1.00 (0.0%)	2.55 (34.3%)	2.60 (38.2%)
Open field + fumigation	CFSD	3.22 (57.7%)	3.77 (77.0%)	3.90 (81.0%)
Open field + fumigation	Disease-free	1.00 (0.0%)	1.60 (9.0%)	1.70 (15%)
Screen house + fumigation	CFSD	2.67 (38.5%)	2.93 (47.6%)	2.20 (33.3%)
Screen house + fumigation	Disease-free	1.00 (0.0%)	1.00 (0.0%)	1.00 (0.0%)

Distribution



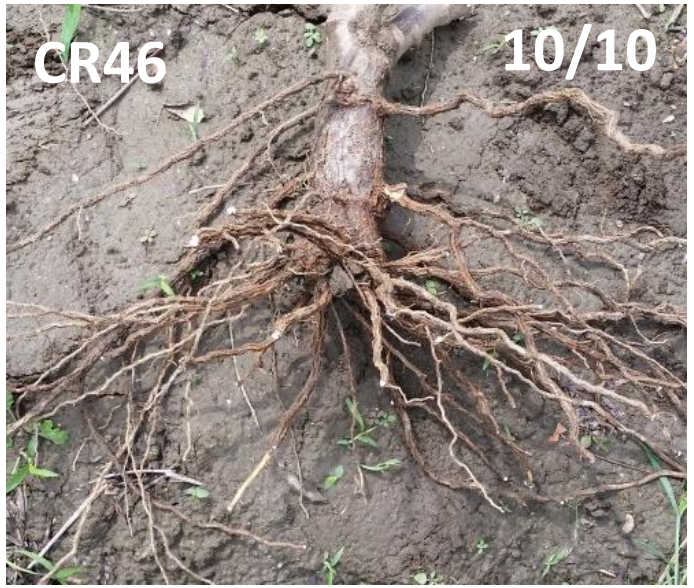
CFSD at CIAT


CMC40
1/10


CR44
10/10

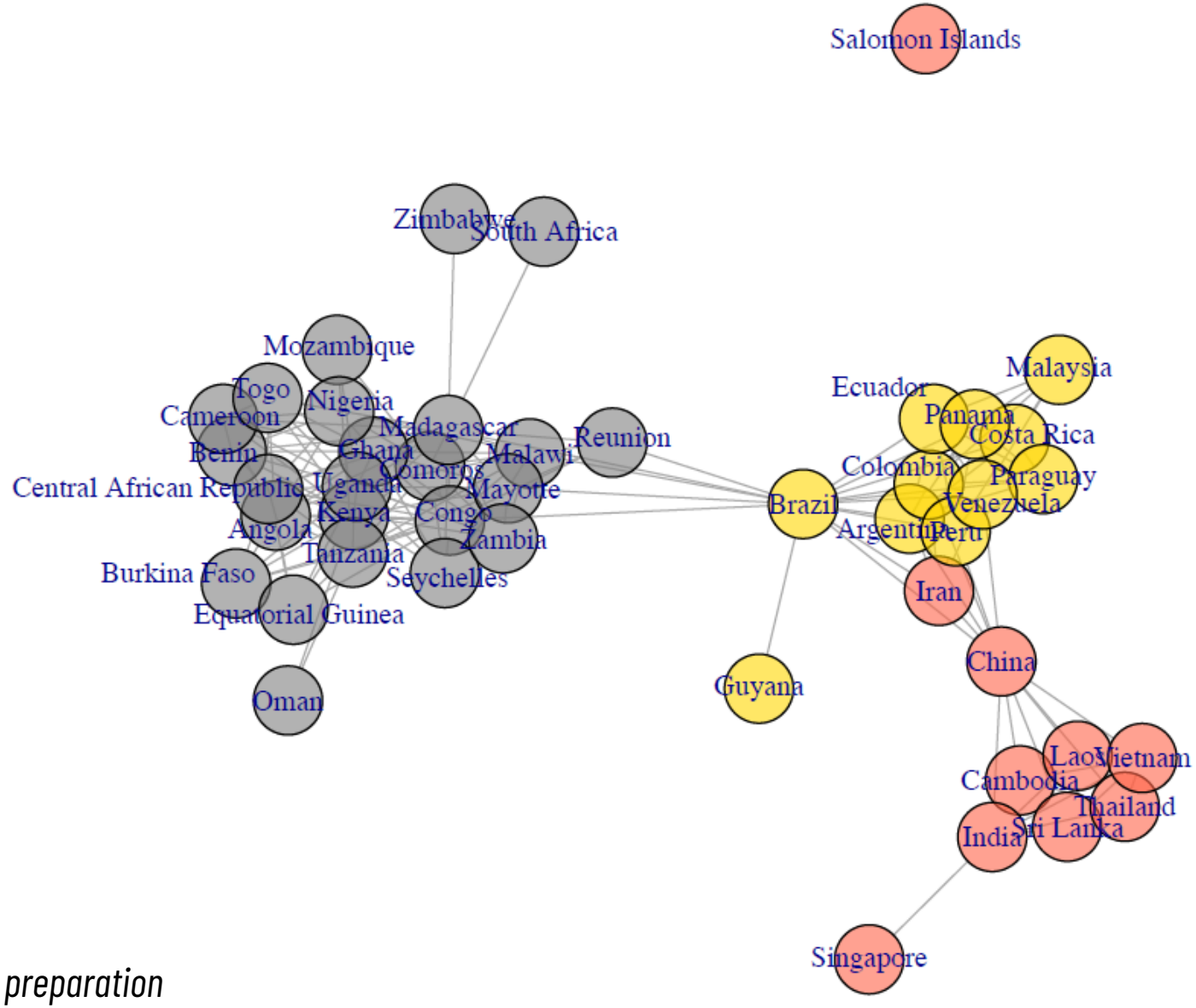

Ecu72
10/10


Bra1A
10/10


CR46
10/10


Per183
0/10

Network analysis – virus x country



Alcalá-Briseno et al., *in preparation*

Field work is where plant pathology begins!

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MethodsX 8 (2021) 101496

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

An optimized nucleic acid isolation protocol for virus diagnostics in cassava (*Manihot esculenta* Crantz.)

Jenyfer Jimenez, Ana Maria Leiva, Cristian Olaya[1], Daniela Acosta-Trujillo, Wilmer Jose Cuellar*

Virology Laboratory, Crops for Nutrition and Health, International Center for Tropical Agriculture (CIAT), AA 6713, Cali, Colombia

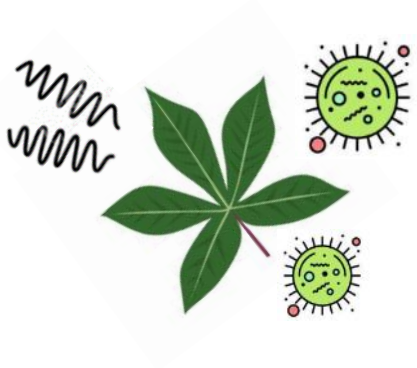
Support projects that involve large scale pathogen surveillance activities

↓

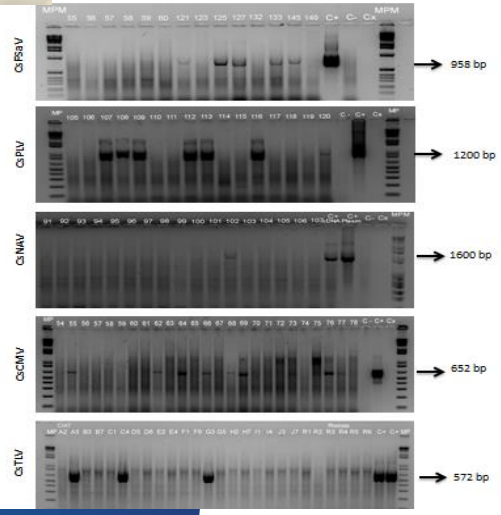
Harmonizing protocols for nucleic acids extraction and field sample collections

↓

Detection and characterization of cassava viruses

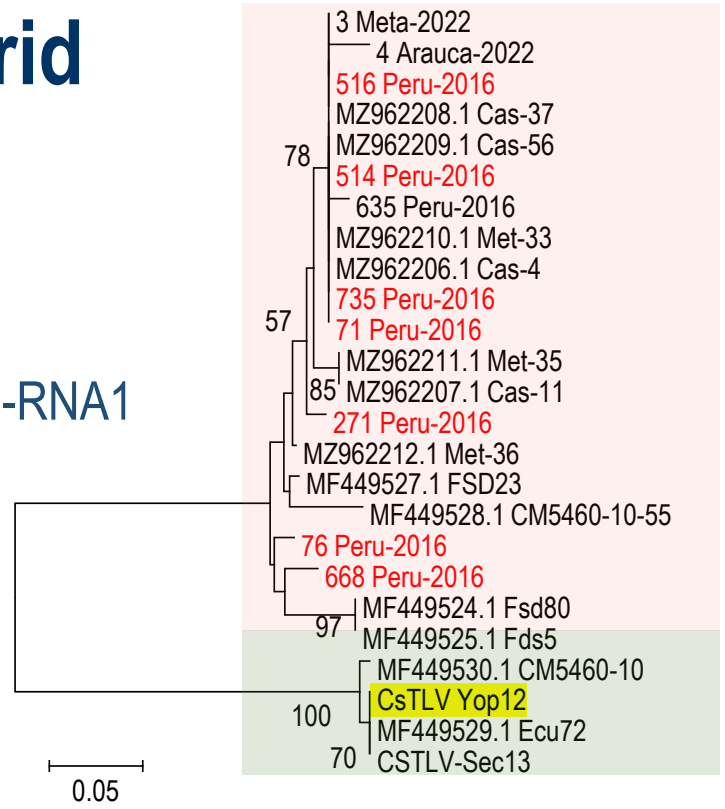


Guarantee consistent results

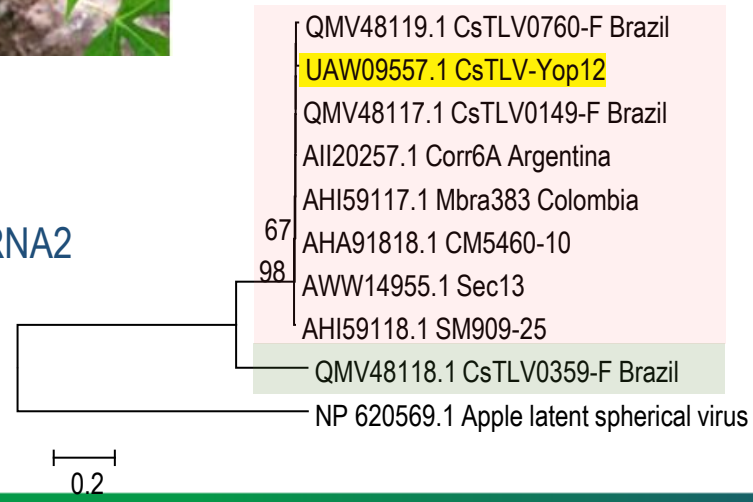


Distribution and diversity of a Secovirid

RdRp-RNA1



CP-RNA2



Phylogenetic trees generated using Neighbor-Joining method and the a.a. sequences of RdRp and CP domain.
The evolutionary distances were computed using the Poisson correction method and are in the units of the number of a.a. substitutions per site.

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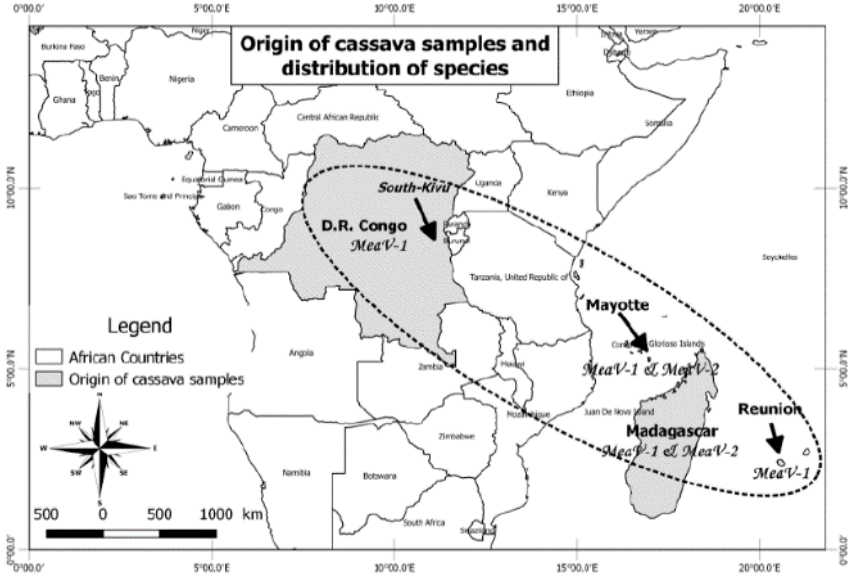
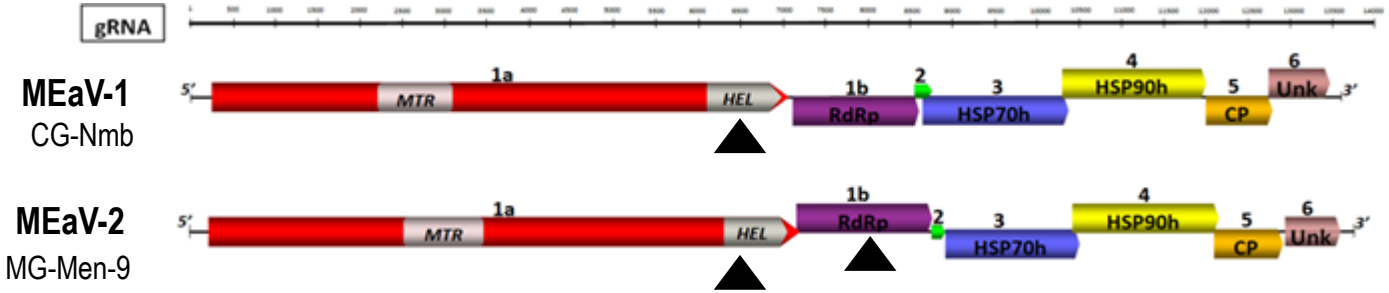
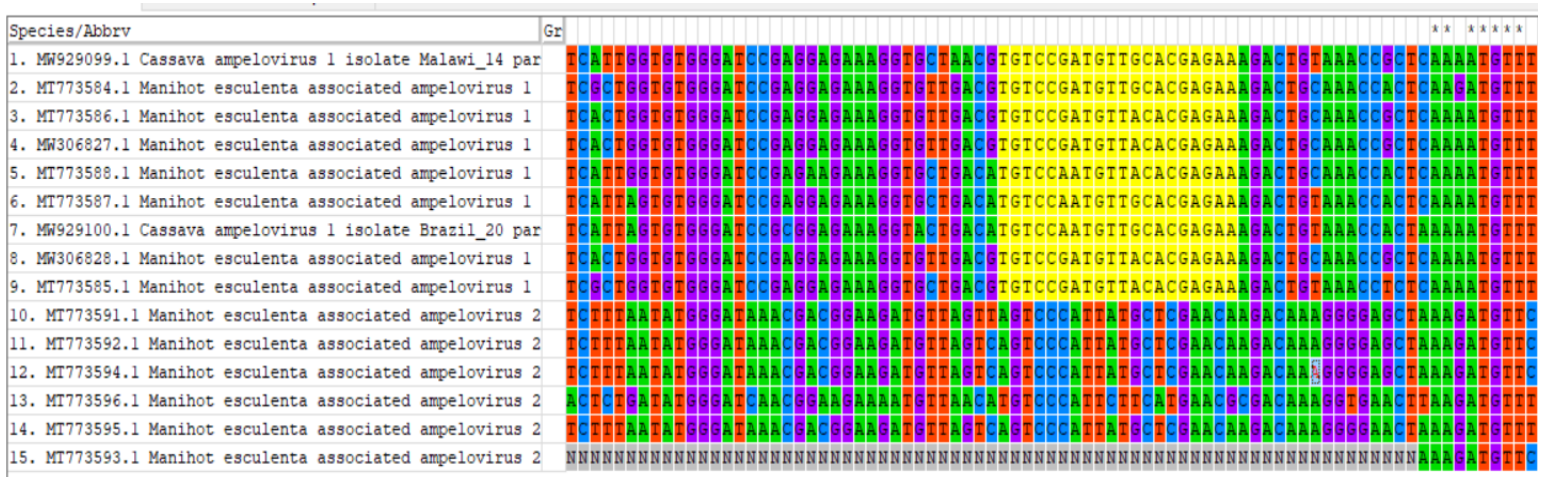
Manihot esculenta-associated virus 1 and 2 (MEaV-1 and MEaV-2)

Field surveys (2016) and germplasm collections (2015-2017)





▲ Location of degenerated primers designed

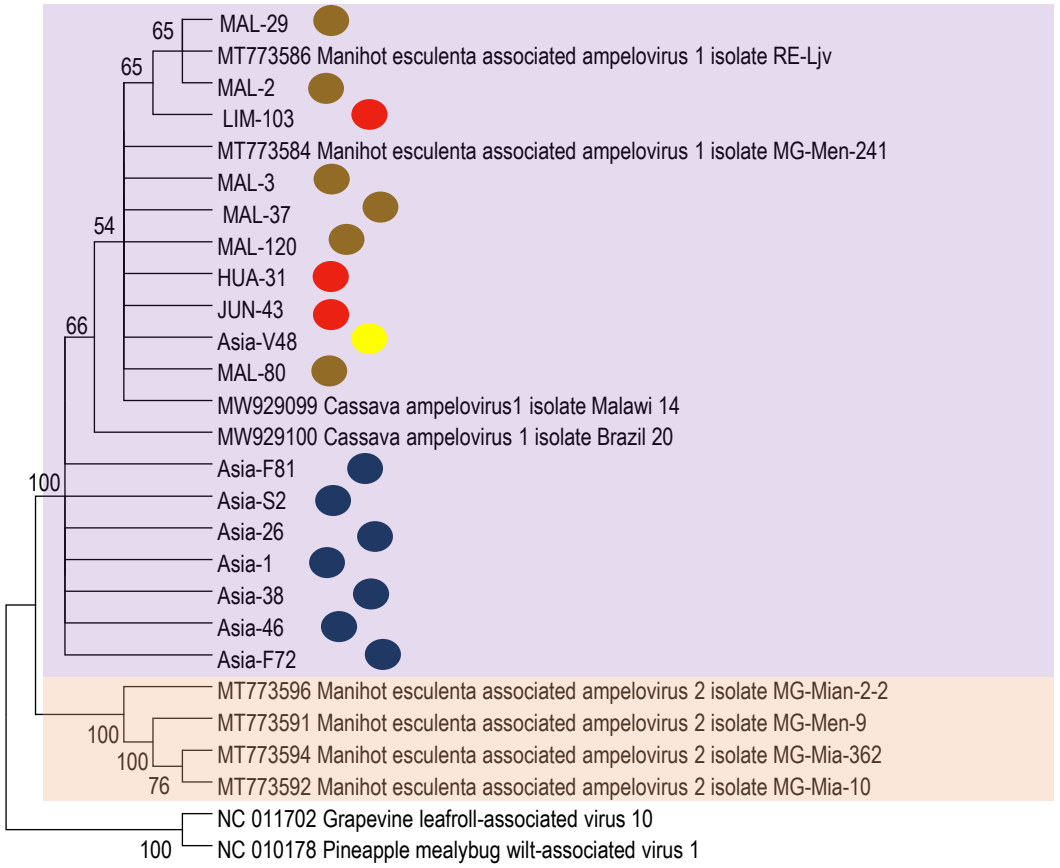


Sequence alignment of MEaV-1 and MEaV-2

Source: Kwibuka et al., (2021)

Phylogenetic analysis of cassava isolates of MEaV-1 and MEaV-2

- Malawi
- Peru
- Vietnam
- Laos



MEaV-1

MEaV-2

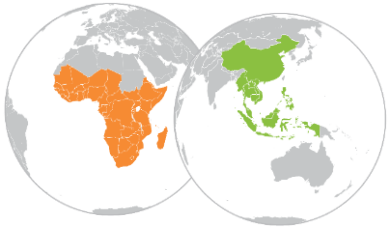
Phylogenetic relationship of ampeloviruses based on the Helicase domain (a.a) of the isolates from Africa, Asia and Latin America.
The evolutionary history was inferred using the Neighbor-Joining method and the distances were calculated using Poisson model.
The tree was generated using MEGA v6.

Cassava Brown Streak Disease (CBSD)

Zhang, Xiaofei

Cassava Brown Streak Disease

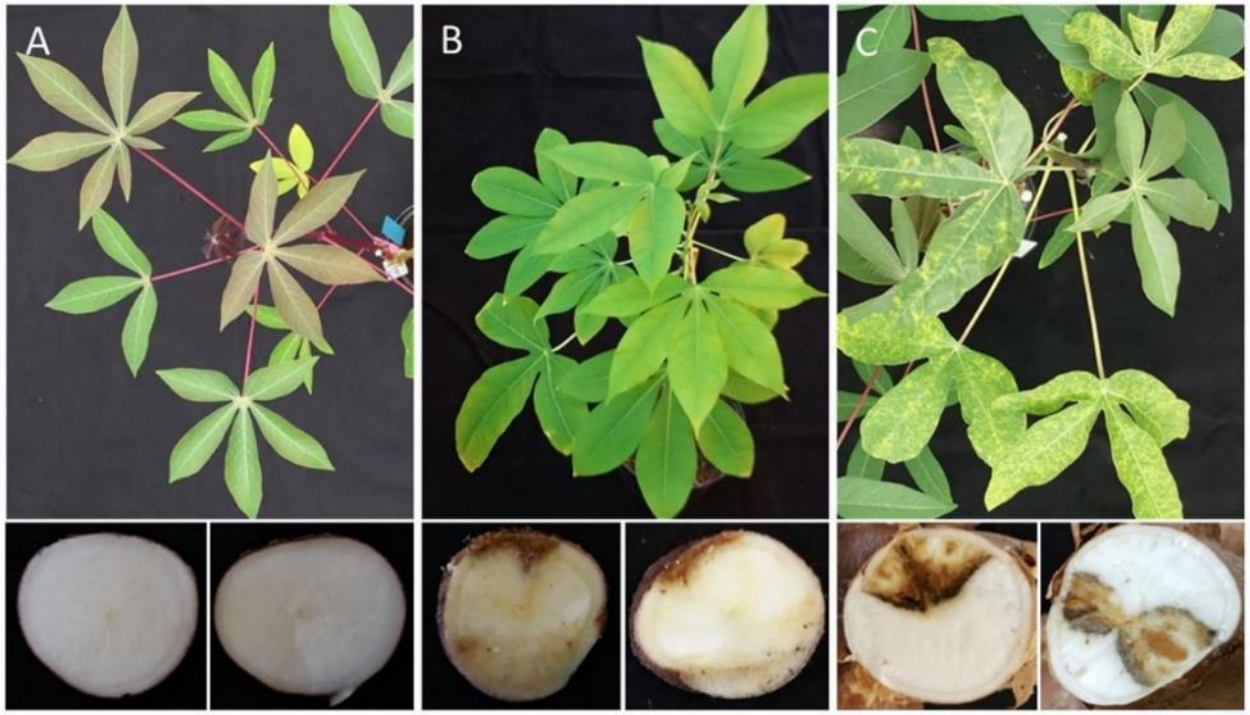
Cassava Mosaic Disease



Cassava Witches' Broom Disease

Cassava Frogskin Disease

Immunity to **CBSV from LAC to SSA**



Breeding populations at ***PYT stage*** in Tanzania and Uganda in 2023.

maternal	paternal	number
PER353	GM7673	4
GM10054B	PER353	6
GM10054B	PER353	8
GM10055B	PER353	3
GM10062	PER353	3
C33	PER353	5
C39	PER353	5
C243	PER353	5
C413	PER353	4
GM10054B	PER221	8
C33	PER221	5
GM6127	PER221	8
GM7672	PER221	15
COL144	GM7673	4
COL144	GM10055B	3
COL144	GM10055B	8
COL144	C33	6
COL144	C39	3
COL144	C19	54
COL144	**C39**	**195**
COL144	TME3	42
KBH2016B	TME14	23
COL40	C33	3
COL40	KBH2016B	17
COL40	KBH2016B	8
COL40	**TME14**	**103**
COL40	GM6127	21
COL40		57
ECU41		8
		634

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IITA Transforming African Agriculture

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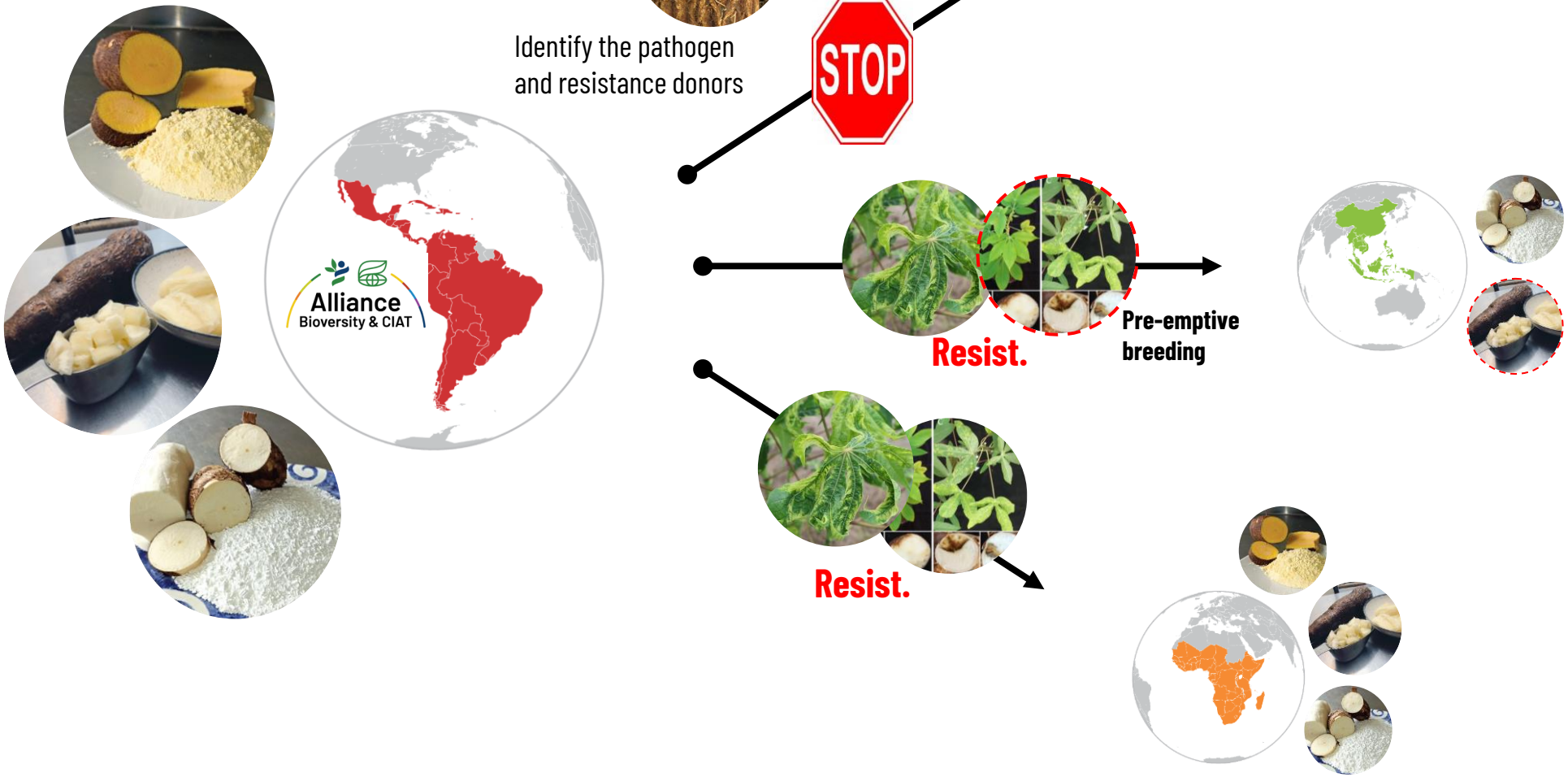
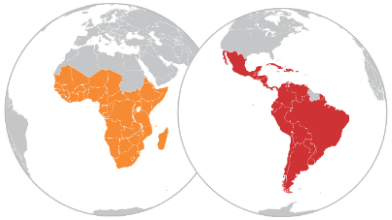
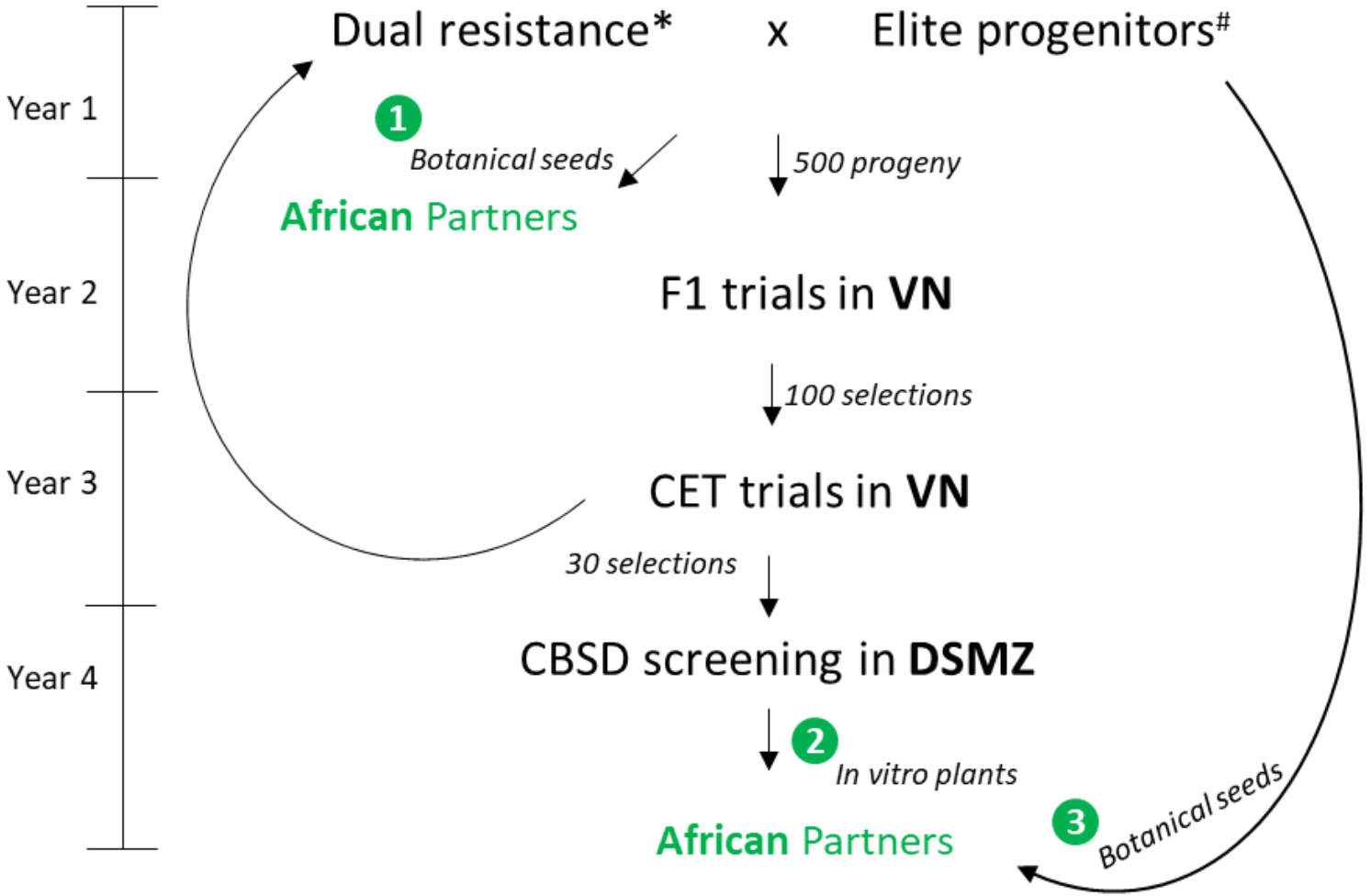

Identify the pathogen
and resistance donors
STOP
Alliance
Bioversity & CIAT
Resist.
Pre-emptive
breeding
Resist.

Identify the pathogen
and resistance donors



Resist.

Resist.



** Dual resistance: resistant to both CBSD and CMD;*
VN, Vietnam; DSMZ, Leibniz Institute DSMZ-German
 # progenitors with erect plant type, high and stable dry matter and high yield

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Thanks!

Questions?

Notes

- Identifying tolerant/resistant varieties against CMD in the current context (i.e. consider CWBD)
- Timely detection of resistance-breaking strains (e.g. African geminiviruses)
- Raise awareness on other cassava transboundary pests and diseases
- Monitoring of whitefly abundance
- Harmonize quarantine and seed movement protocols
- Establish disease models (biological and epidemiological) for risk and impact analyses
- Access to Biosafety Level 2-3 facilities?