Establishing sustainable solutions to cassava disease in mainland Southeast Asia

Objective 3: Develop and deploy diagnostic protocol, tools and information platforms fit for purpose in monitoring, surveillance, and certification applications

Presented by **Le Thi Hang**
Plant Protection Research Institute (PPRI - Vietnam)
Email: Hanglee.ppri@gmail.com
Project final review
3 October 2023

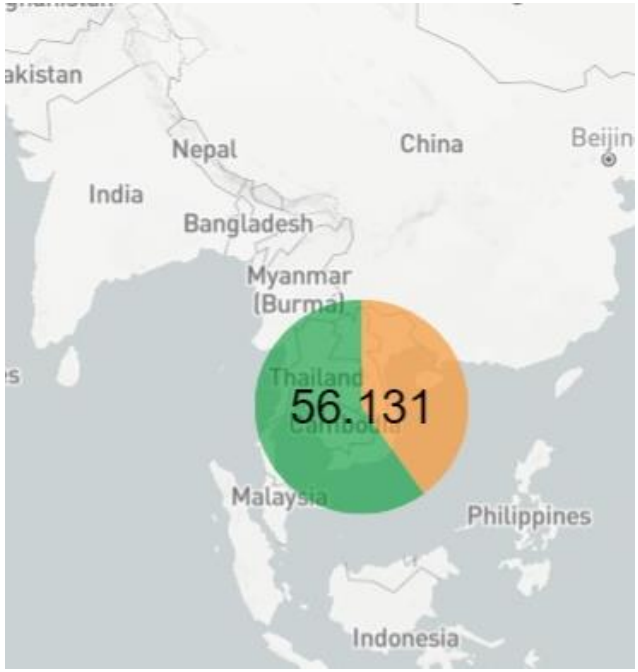
Develop and deploy diagnostic protocol, tools and information platforms fit for purpose in monitoring, surveillance, and certification applications

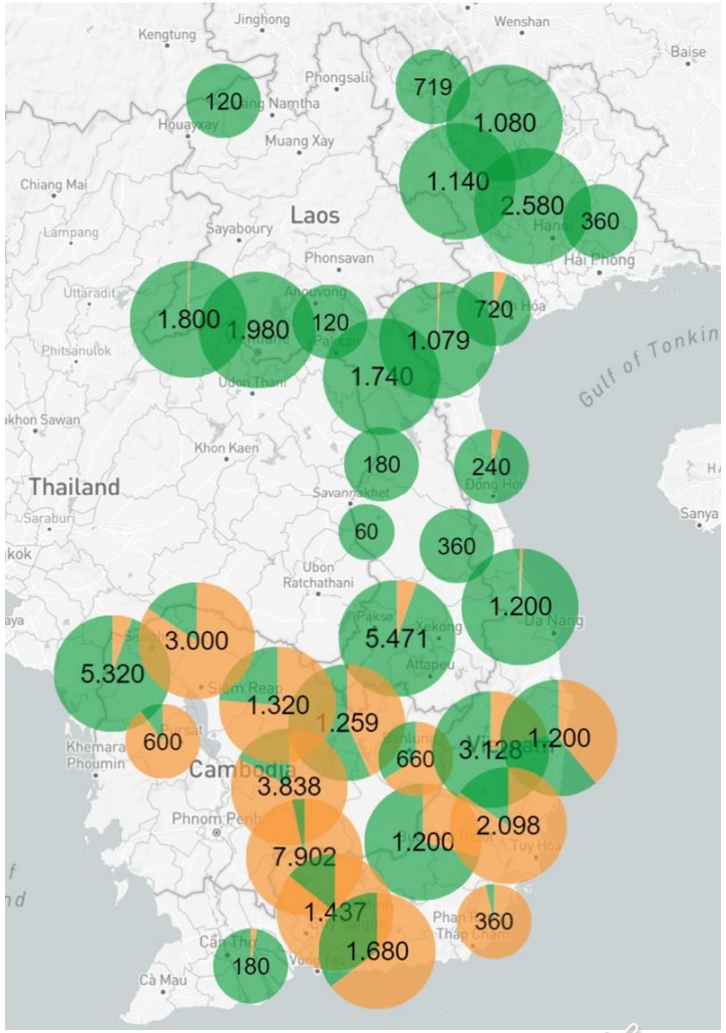
- Conduct training and capacity building of scientists/staff in the plant production institutes in key diagnostic tools, sampling design, and data management platforms
- Design and implement regional surveillance for CMD and CWBD in Vietnam, Cambodia, Lao PDR, Myanmar and Thailand with results shared in a common platform
- Understand the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam, Cambodia, Lao PDR, Myanmar, and Thailand
- Evaluate new technologies of rapid field diagnostic for CMD and CWBD with particular applications in seed systems
- Develop protocols for screening cassava clones and the biological characterization of diseases, particularly CWBD
- Develop and evaluate the effectiveness of communication products and awareness raising strategies utilising different public and private sector stakeholders

Surveillance 2020-2022

CASSAVA MOSAIC DISEASE (CMD) and *CASSAVA WITCHES' -BROOM DISEASE* (CWBD)

- ✓ **37,980** observations were shared on PestDisPlace that were conducted by PPRI teams during 3 years



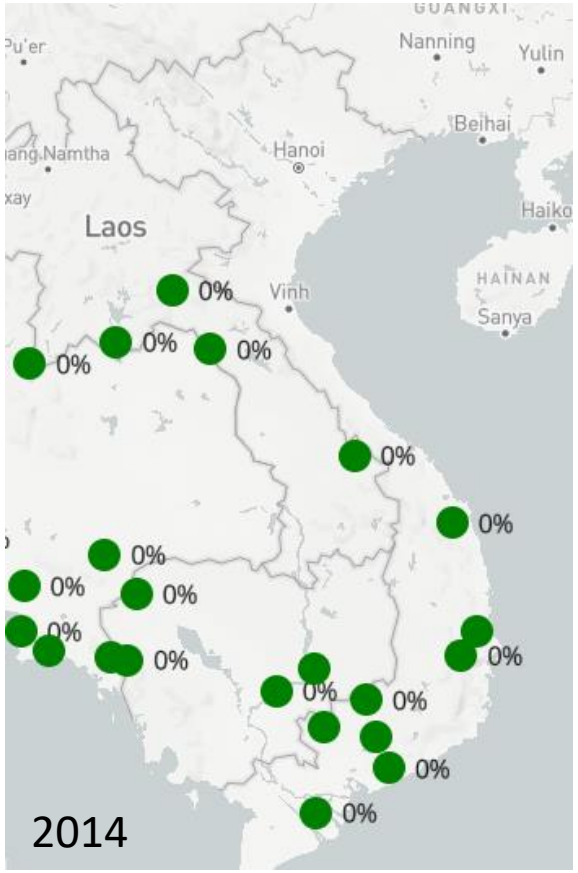


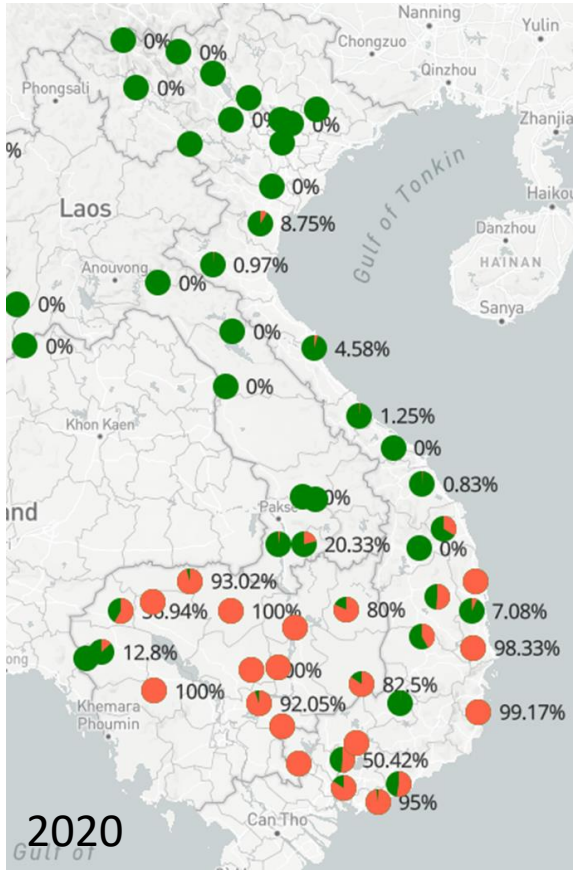
Incidence Maps of CMD

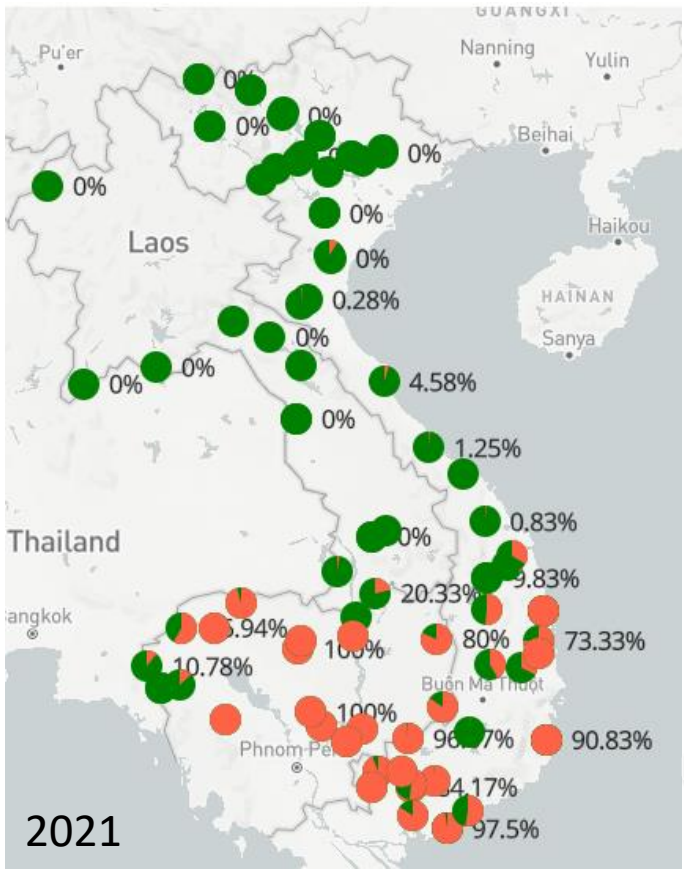
In the three years from 2020 to 2022, a total of **633 fields** were surveyed

The hotspots of the disease are in the *southern*, *central highland*, and *south-central coast*

It has rapidly spread to the central and northern provinces in just 3 years

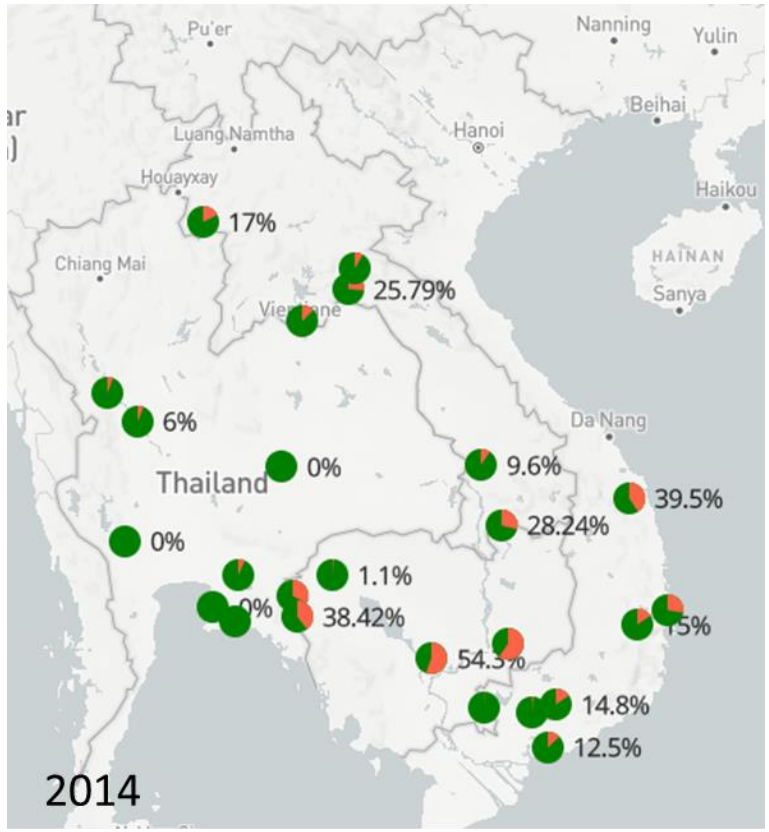


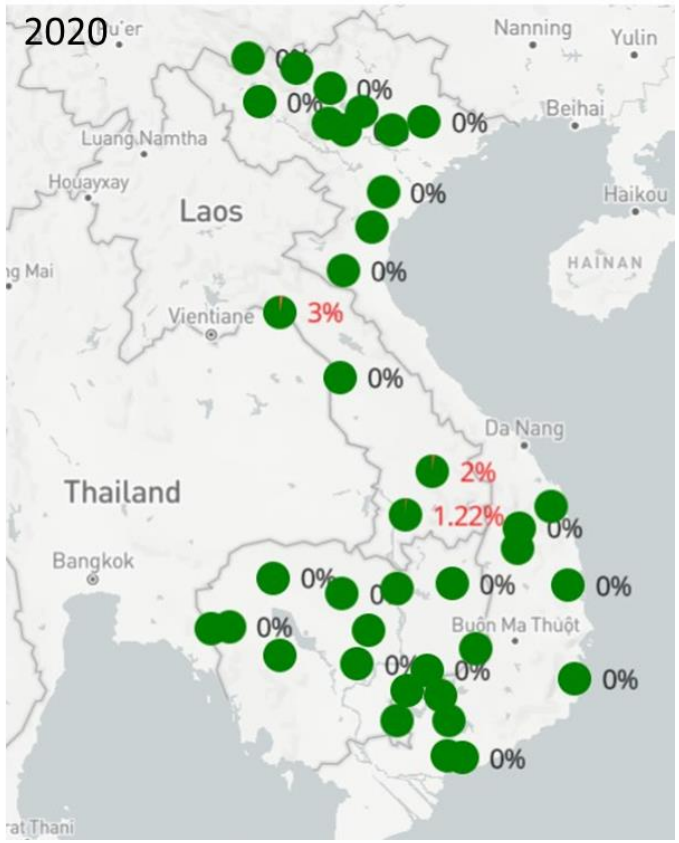


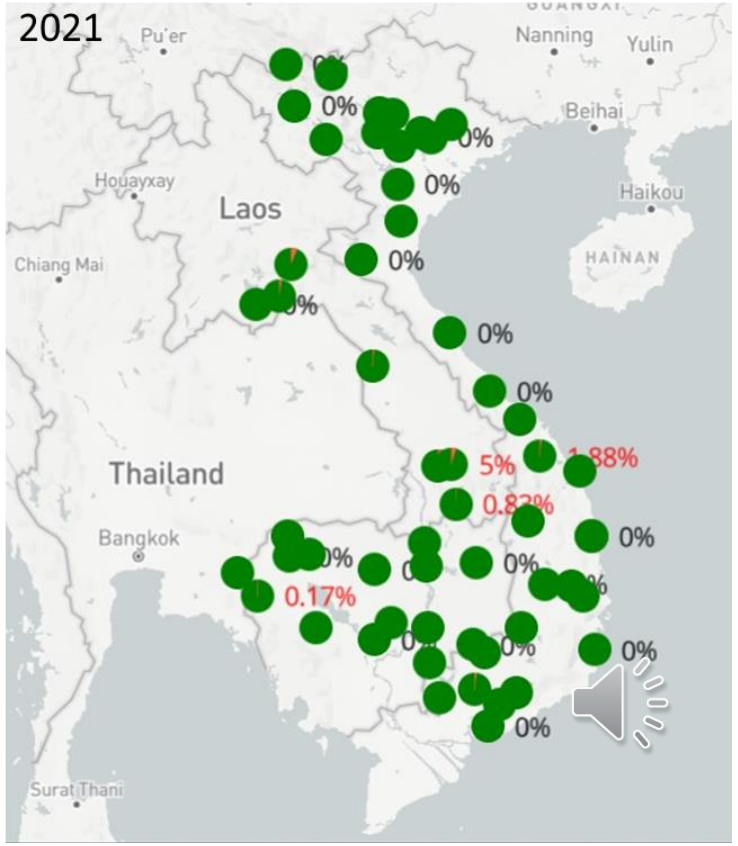


Incidence Maps of CWBD

"First wave" of CWBD in Vietnam in 2010-2015.
Field surveys in 2020 - 2021 identified 1 location.
In 2022, no symptoms were detected during the sampling process (however, they were subsequently found in sideline activities).
In 2023, CWB symptoms were recorded in many cassava cultivation regions.

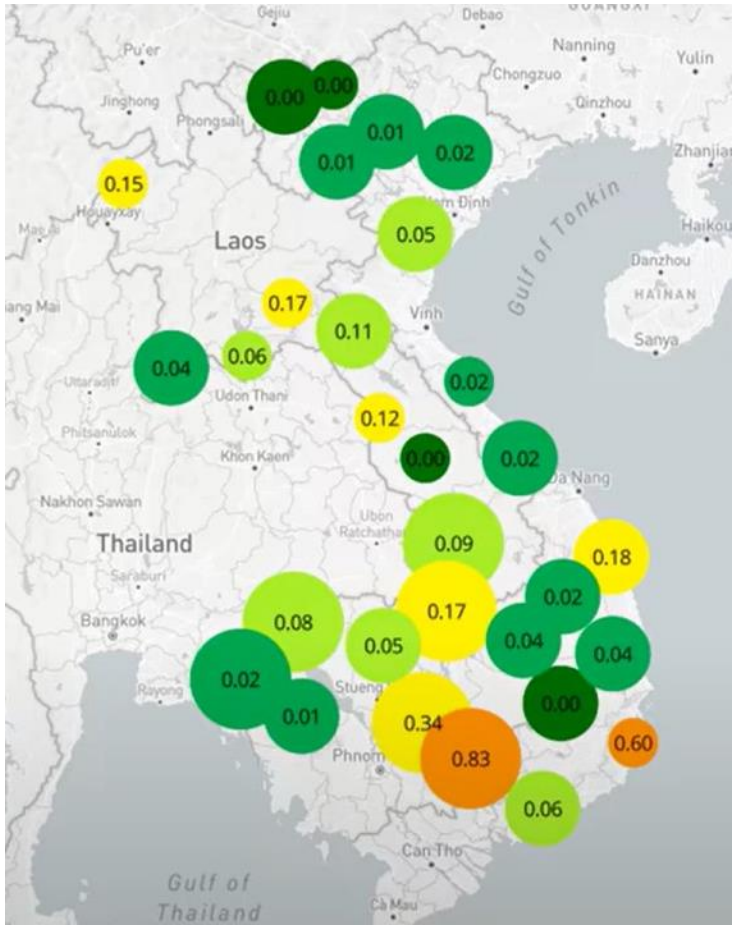


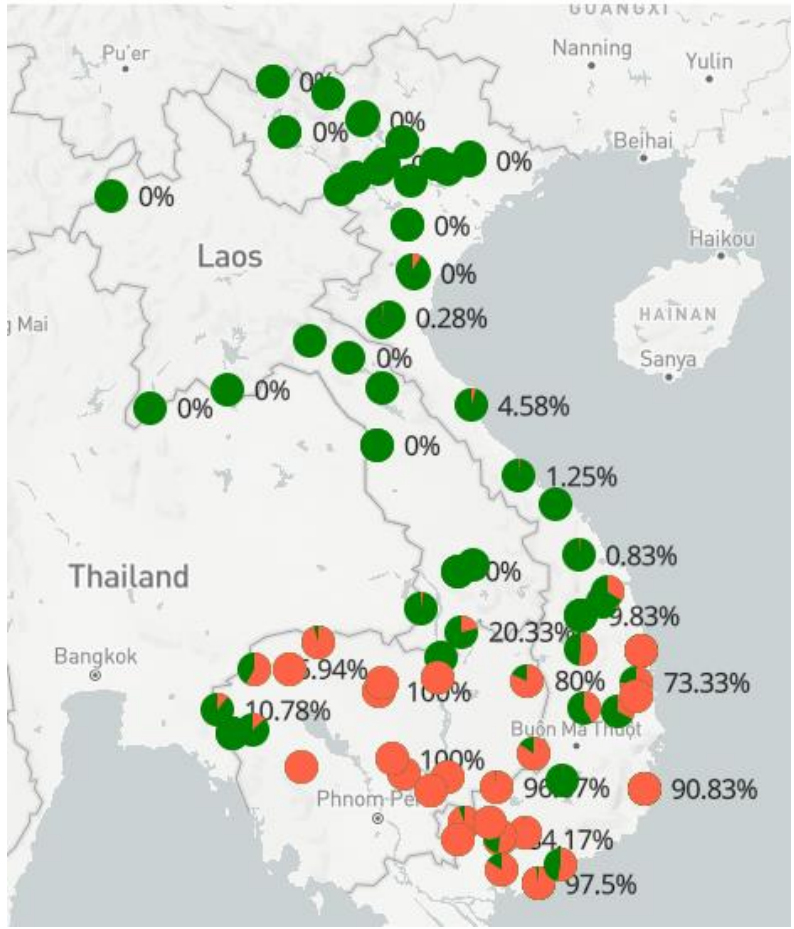




Incidence Maps of Whitefly

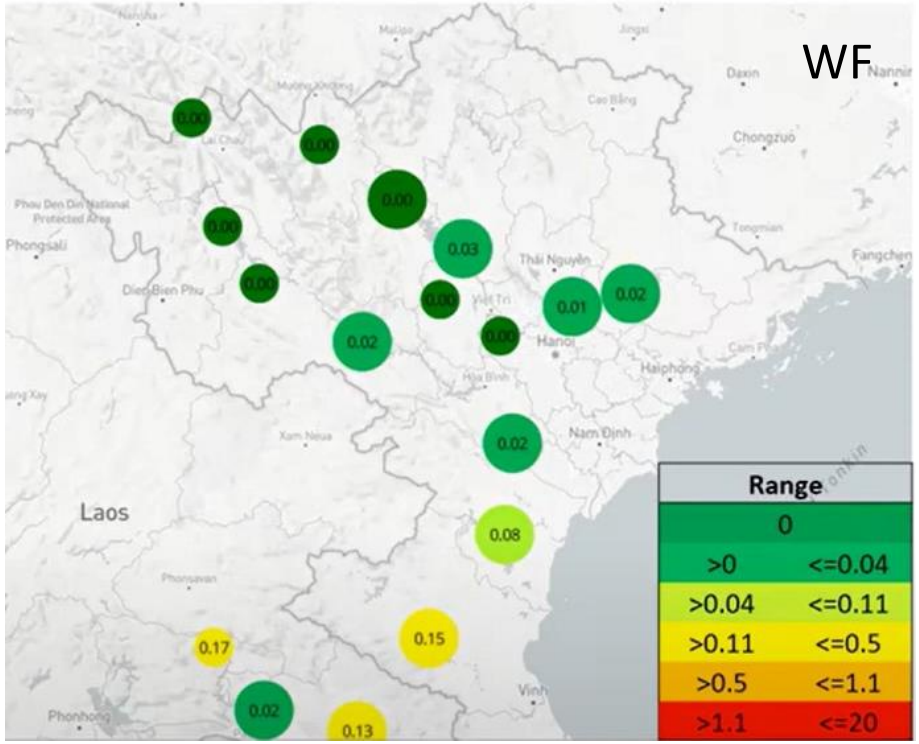
Incidence in the southern, central highland, and central coast regions is higher than those in the north.
Regions that are severely affected by CMD tend to have higher whitefly incidence.
=> The correlation with the CMD
=> The transmission of CMD through **whiteflies** and **planting materials**

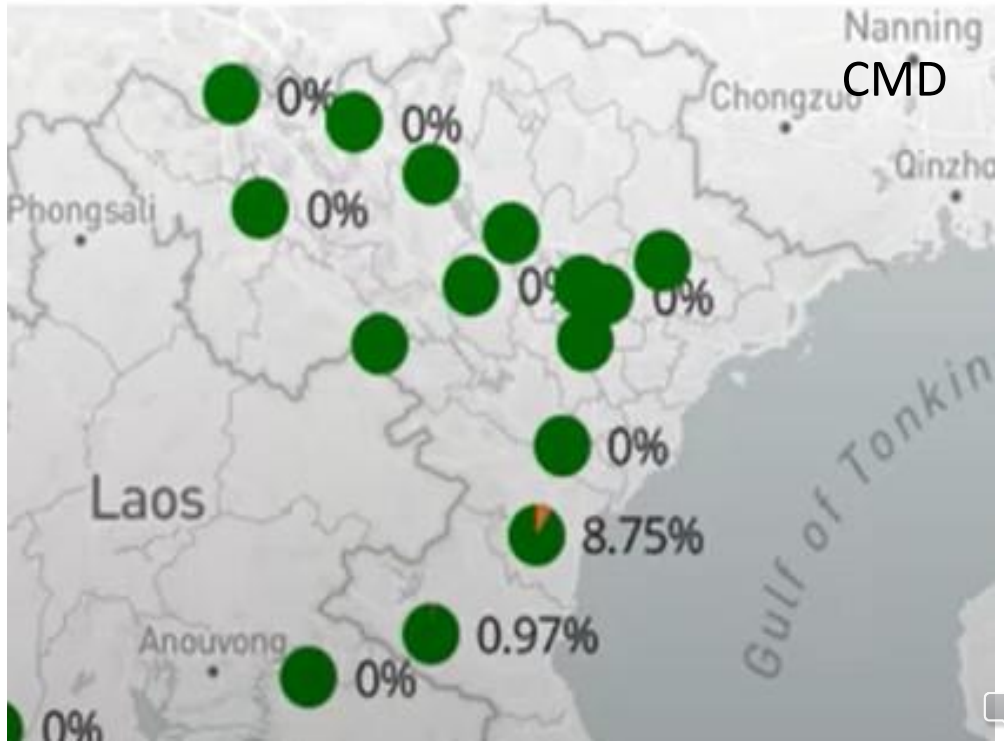




Whitefly relative abundance 2020-2022

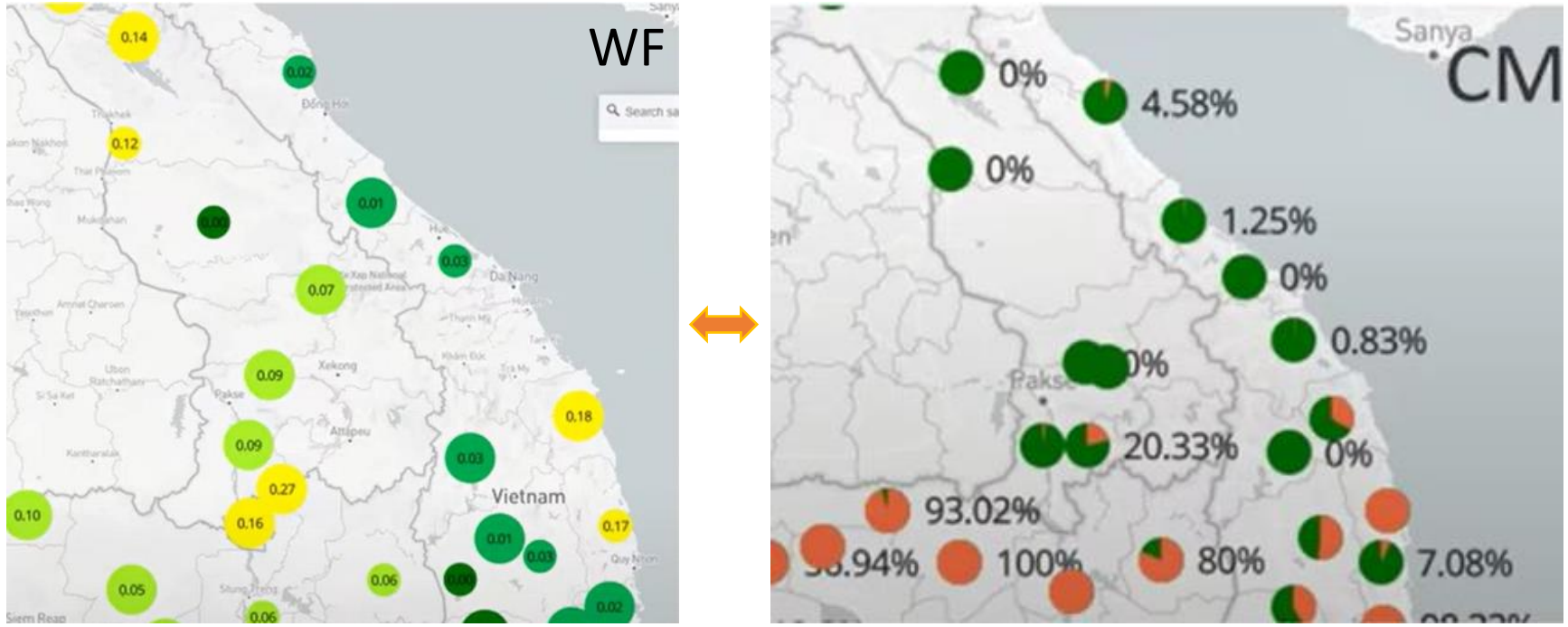
Incidence in the southern, central highland, and central coast regions is higher than those in the north.
Regions that are severely affected by CMD tend to have higher whitefly incidence.
=> The correlation with the CMD
=> The transmission of CMD through **whiteflies** and **planting materials**





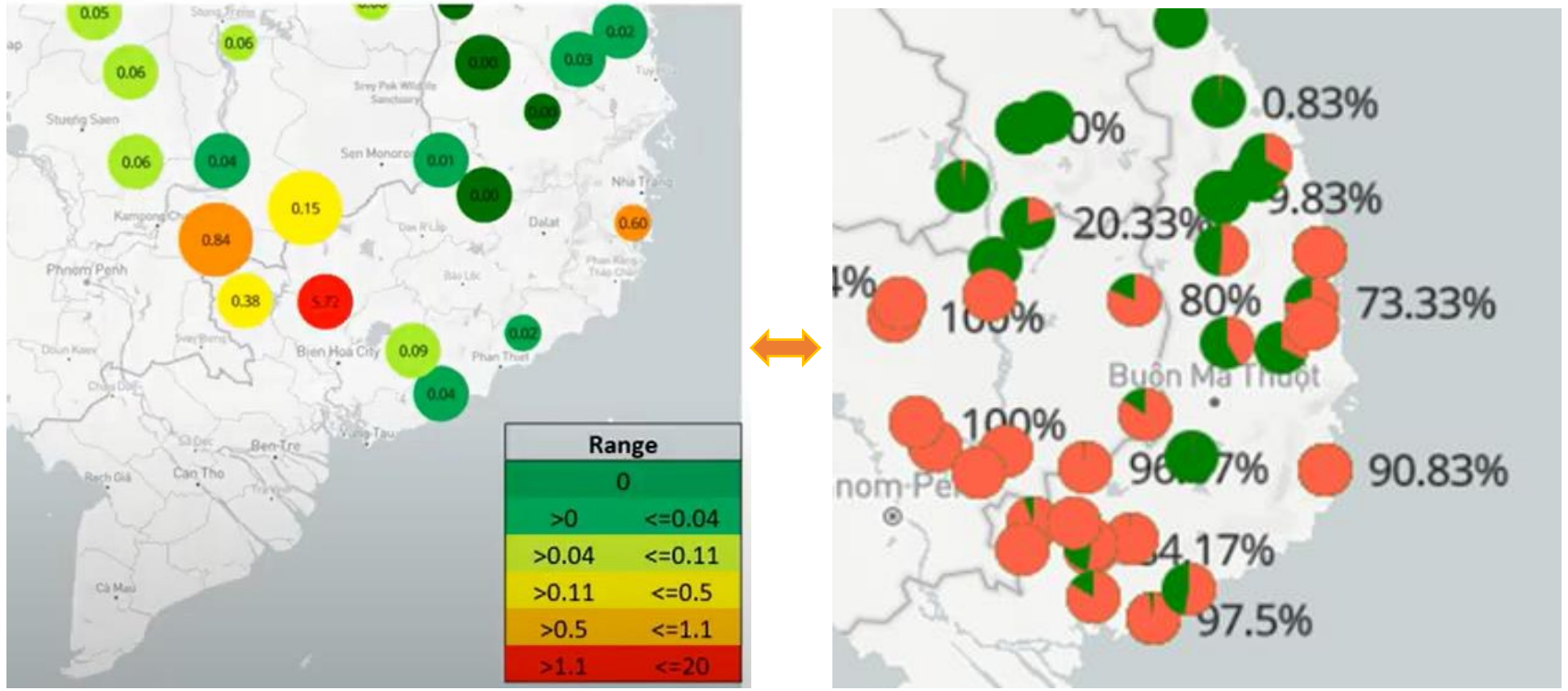
Whitefly relative abundance 2020-2022

Incidence in the southern, central highland, and central coast regions is higher than those in the north.
Regions that are severely affected by CMD tend to have higher whitefly incidence.
=> The correlation with the CMD
=> The transmission of CMD through **whiteflies** and **planting materials**



Whitefly relative abundance 2020-2022

Incidence in the southern, central highland, and central coast regions is higher than those in the north.
Regions that are severely affected by CMD tend to have higher whitefly incidence.
=> The correlation with the CMD
=> The transmission of CMD through **whiteflies** and **planting materials**



Understanding the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam

1. Sampling of whiteflies designed during surveys

2. Update data (photos) of whitefly density sampling fields.

3. Evaluate the correlation between the cassava leaves and whiteflies in Vietnam by determining the density of whiteflies on the first 5 youngest leaves

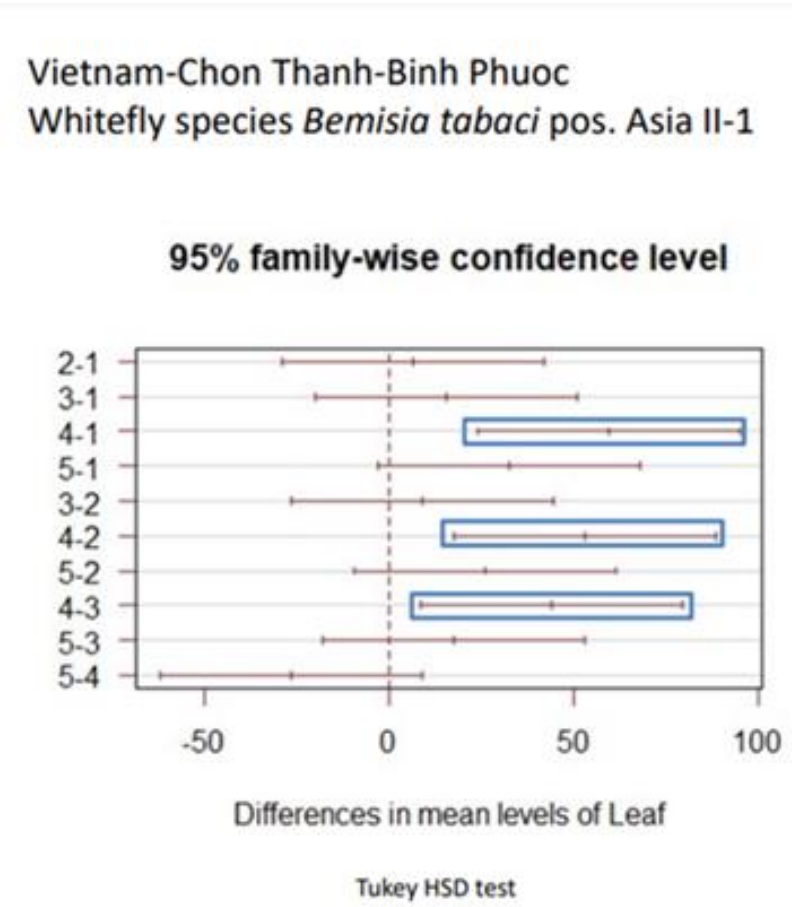
4. Sequencing of 73 whitefly samples collected during 2020-2021 surveys for phylogenetic analysis based on mtCOI gene to sequence characterize the identify a different populations.and identify a different population

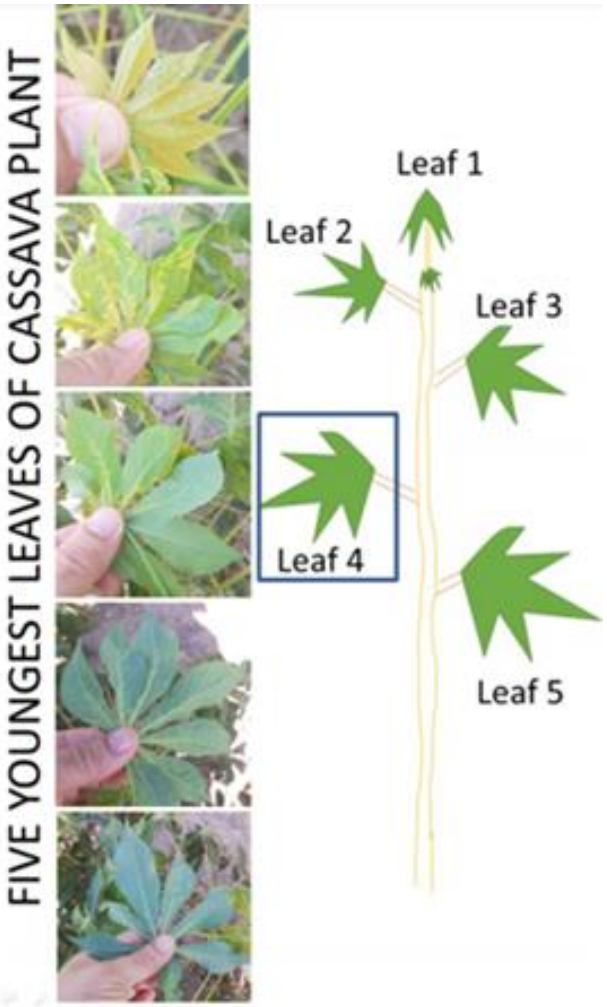
Understanding the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam

- ✓ Determine the density of whiteflies by taking a picture of the first fully expanded leaf and then collecting whiteflies for diversity analysis in SEA.

- ✓ Evaluate the correlation between the cassava leaves and whiteflies in Vietnam by determining the density of whiteflies on the first 5 youngest leaves from 30 plants from different fields

=> The preference of WF is **the fourth leaf**

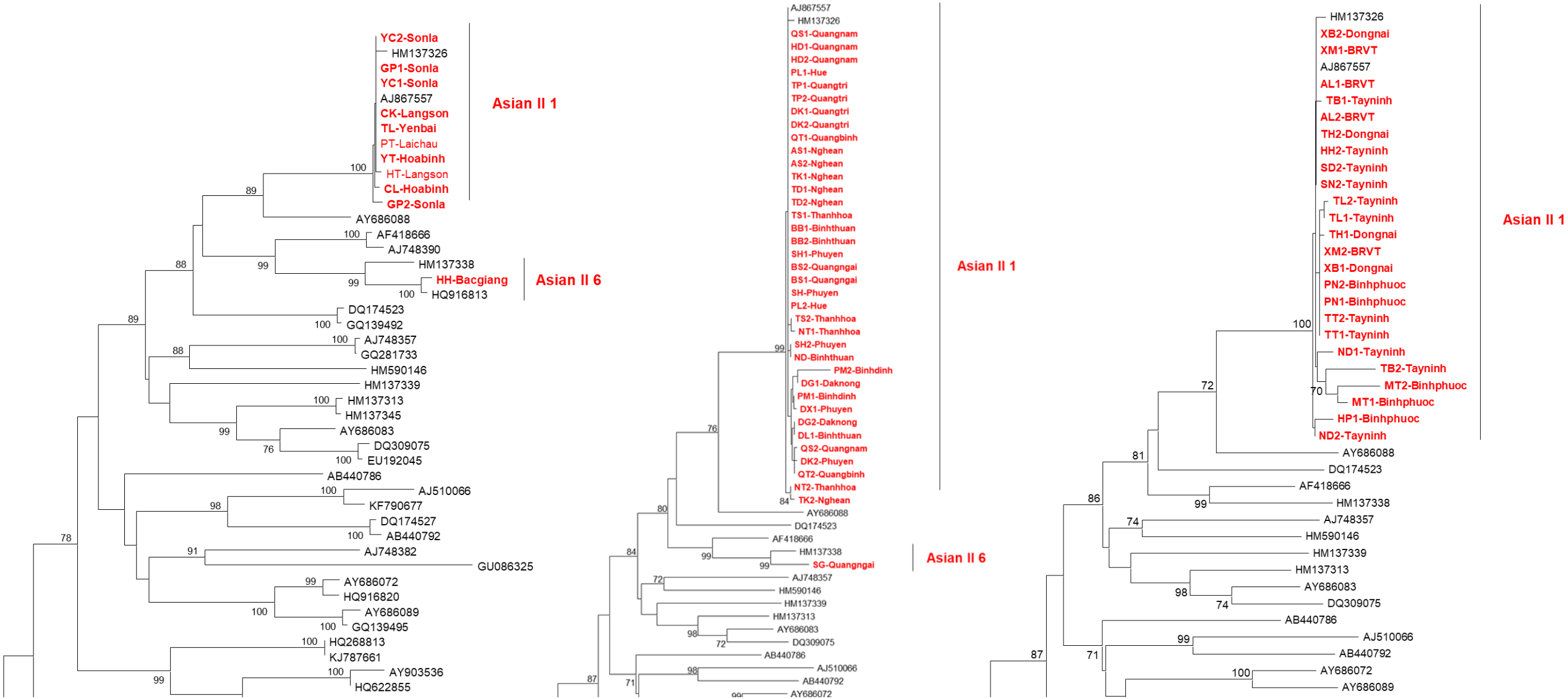




Understanding the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam

Sequencing of 73 whitefly samples collected during 2020-2021 surveys for phylogenetic analysis based on mtCOI gene to sequence characterize the identity and different populations.

Understanding the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam



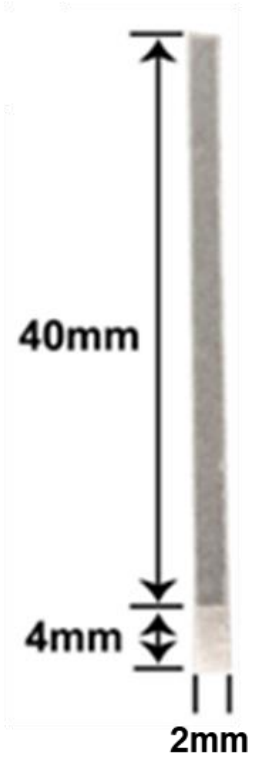
Understanding the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam

Sequencing of 73 whitefly samples collected during 2020-2021 surveys for phylogenetic analysis based on mtCOI gene to sequence characterize the identity and different populations.

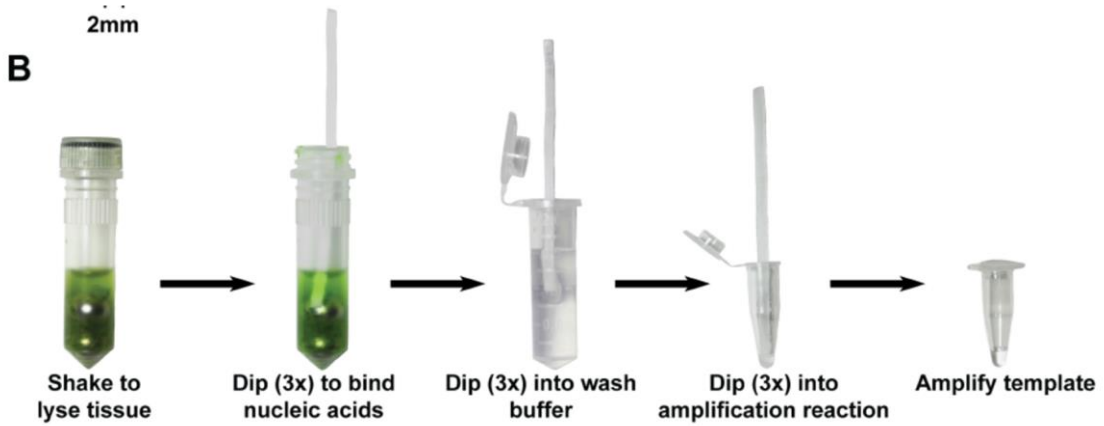
Location	No. of samples	Biotype
Northern zone	11	Asian II 1, Asian II 6
Middle zone	38	Asian II 1, Asian II 6
Southern zone	24	Asian II 1

Evaluate new technologies of rapid field diagnostic for CMD

Evaluate new technologies of rapid field diagnostic for CMD



Dipstick



DNA extraction

Portable assay device

CWBD IN VIETNAM 2023

CWBD activities 2023

- ✓ **Surveillance activities for CWBD in cassava growing region Vietnam**

- ✓ **Detection of agents on CWBD**

In 2010

Cassava Witches Broom in Quang Ngai Province, Vietnam

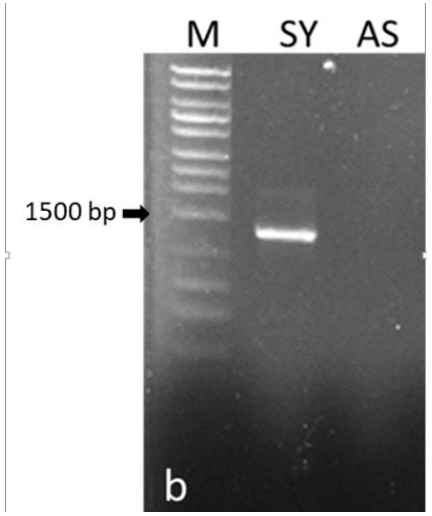
PHYTOPLASMA

Map of clustered sampling points for CWB detected in Viet Nam in 2010

*Field surveillance was carried out in late April **2021** in the southern province - Kontum.*
The result has notably shown that CWB was coming back after a long absence.

Map of clustered sampling points for CaWB detected in Viet Nam in 2021

P1/P7-
R16F2n/R16R2



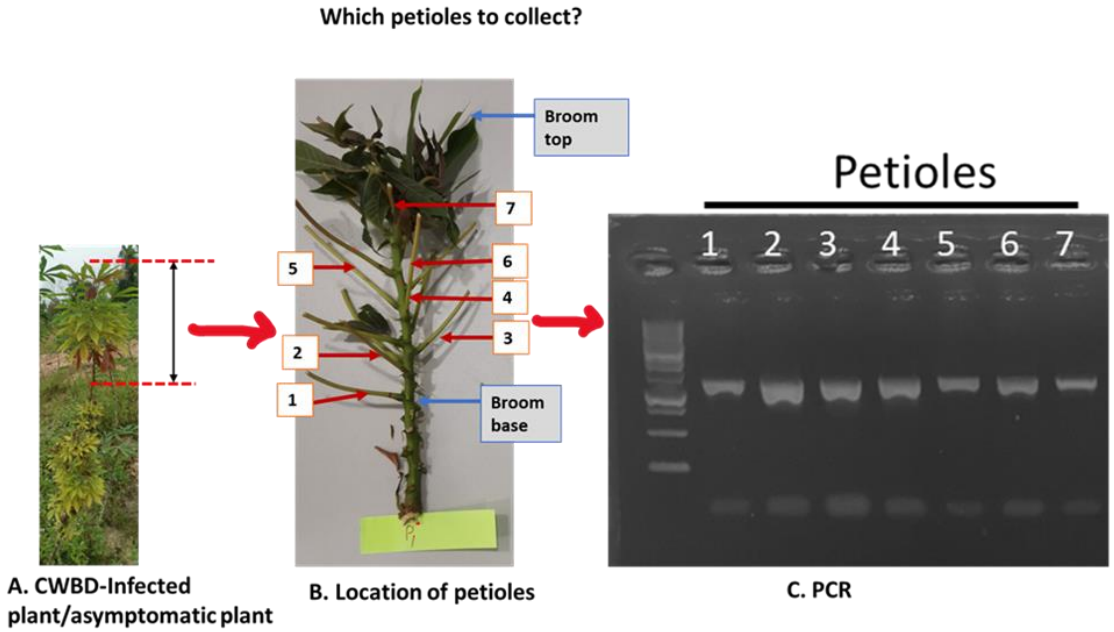
Detection of CWBD through nested PCR using P1/P7 and R16F2N/R16R2

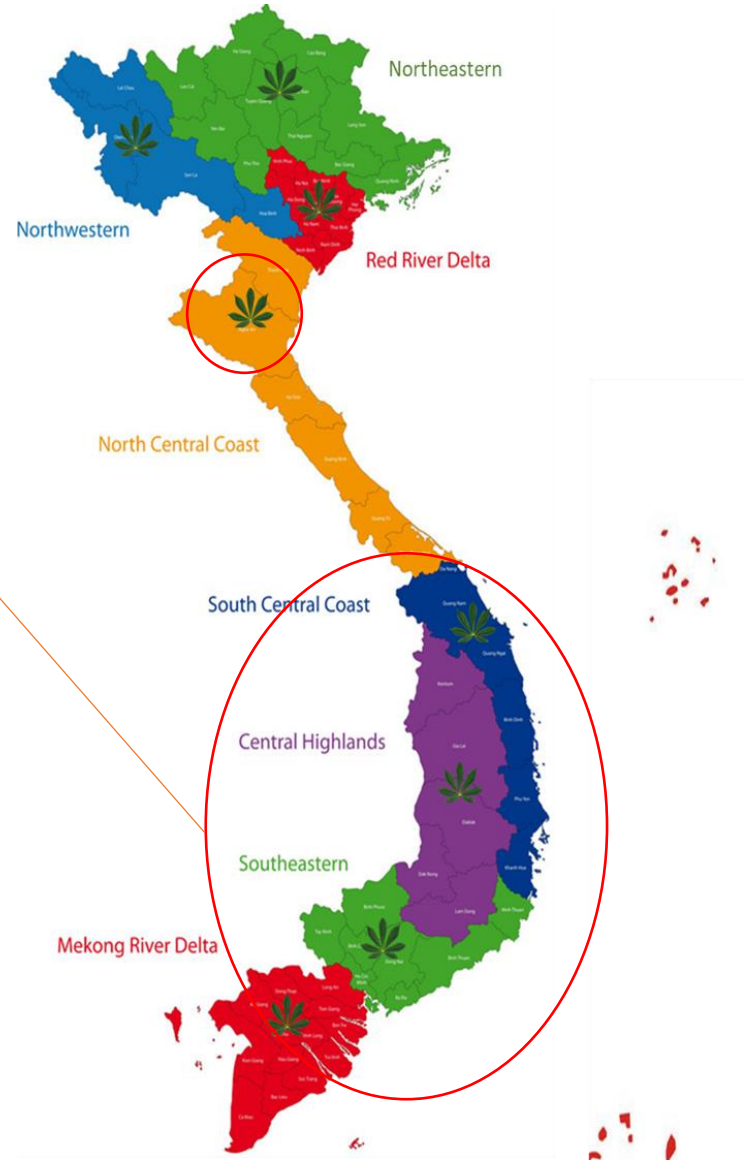
→ ???

Sign of disease

Surveillance activities

- *Field survey and sampling in 7 southern growing cassava provinces (Kontum, DakLak, Gia Lai, Tay Ninh, Dong Nai)*
- *Sampling protocol by CIAT's*

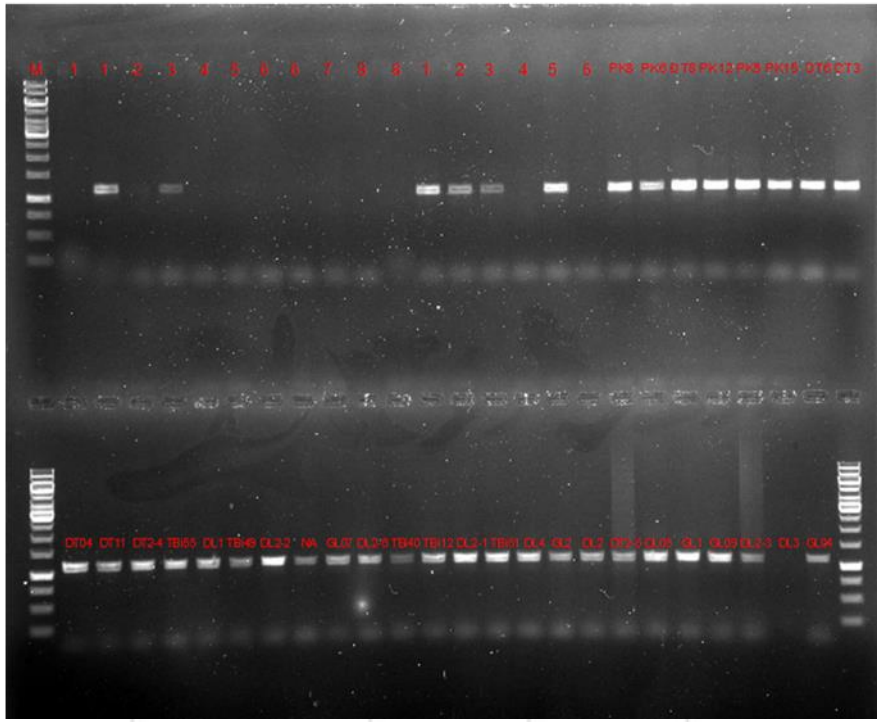




Detection of agents on CWBD cassava

Check the accuracy of the Cerato primer

~1,1 kb



blast.ncbi.nlm.nih.gov/Blast.cgi

Job Title	H1 - CeratoF2R2
RID	91S73WZU013 *Search expires on 06-21 07:12 am* Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_34133
Description	H1 - CeratoF2R2
Molecule type	dna
Query Length	1046
Other reports	Distance tree of results MSA viewer

Filter Results

Organism *only top 20 will appear* exclude

Type common name, binomial, taxid or group name

+ Add organism

Percent Identity to | E value to | Query Coverage to

Filter Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Select columns Show 100

select all *6 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhizoctonia solani CAMK/CAMK1 protein kinase (RhiXN_01588), partial mRNA	Rhizoctonia solani	216	216	58%	1e-50	67.39%	5286	XM_043321407.1
Rhizoctonia solani strain AG-1 IA isolate XN chromosome 16	Rhizoctonia solani	216	216	58%	1e-50	67.39%	3605667	CP059673.1
Rhizoctonia solani strain AG1-IA_HG81 chromosome 16	Rhizoctonia solani	212	212	58%	1e-49	67.24%	3837216	CP102658.1
Ceratobasidium sp. AG-Ba isolate JN chromosome 32	Ceratobasidium sp. AG-Ba	66.2	66.2	8%	1e-05	77.01%	3814631	CP059641.1
Ceratobasidium sp. AG-Ba isolate LY chromosome 16	Ceratobasidium sp. AG-Ba	57.2	57.2	8%	0.007	74.71%	3695994	CP059657.1
Ceratobasidium sp. AG-Ba isolate JN chromosome 16	Ceratobasidium sp. AG-Ba	57.2	57.2	8%	0.007	74.71%	3729354	CP059625.1

	No of samples	PCR +	PCR -	Accuracy rate (%)	sensitivity[TP/(TP+FN)*100]	specificity [TN/(TN+FP)*100]
Symptomatic	47	39	8	83	83	100
Asymptomatic	5	0	5	100		
Unclear symptoms	2	2	0			
Total	54	41	13			

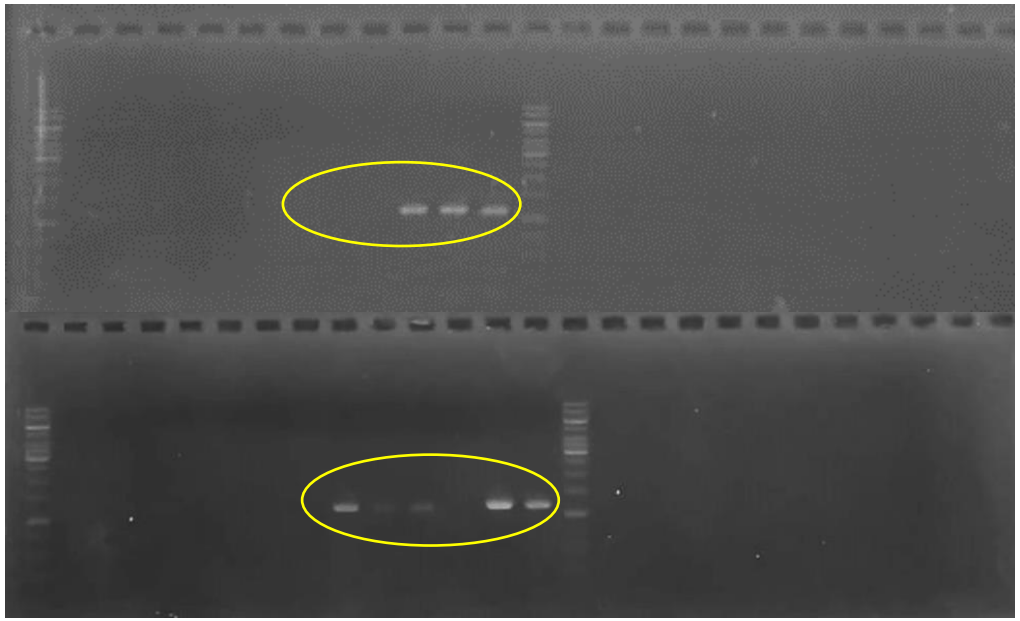
TP=True positives, FP= False negatives, TN= True negatives, FN= False postives

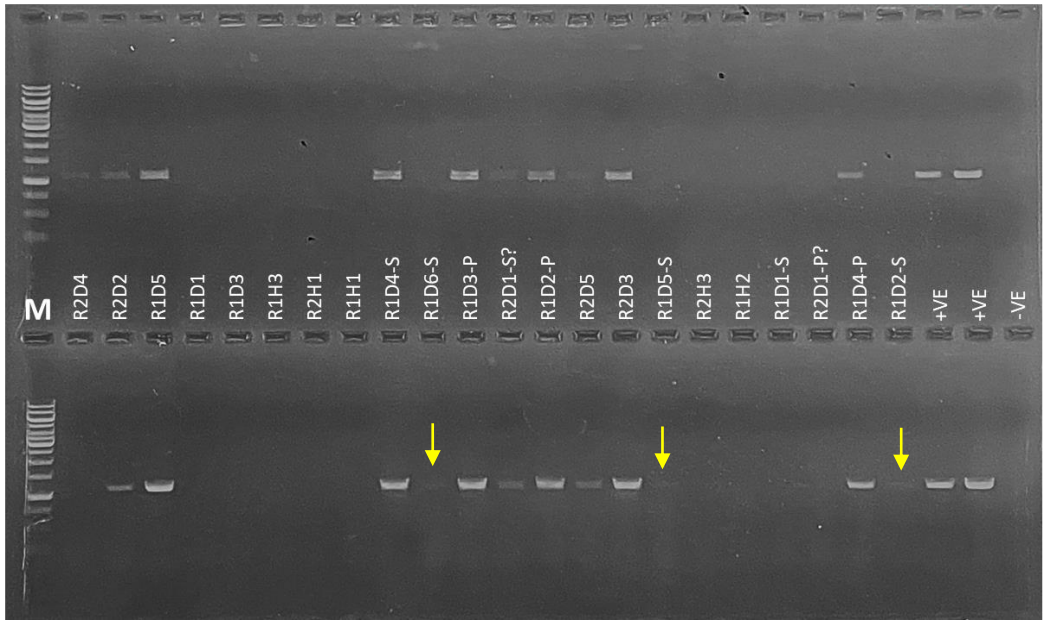
Detection of agents on CWBD cassava

Check the accuracy of pairs of primer detecting Ceratobasidium

CeratoF2/R2

BJF2/R2





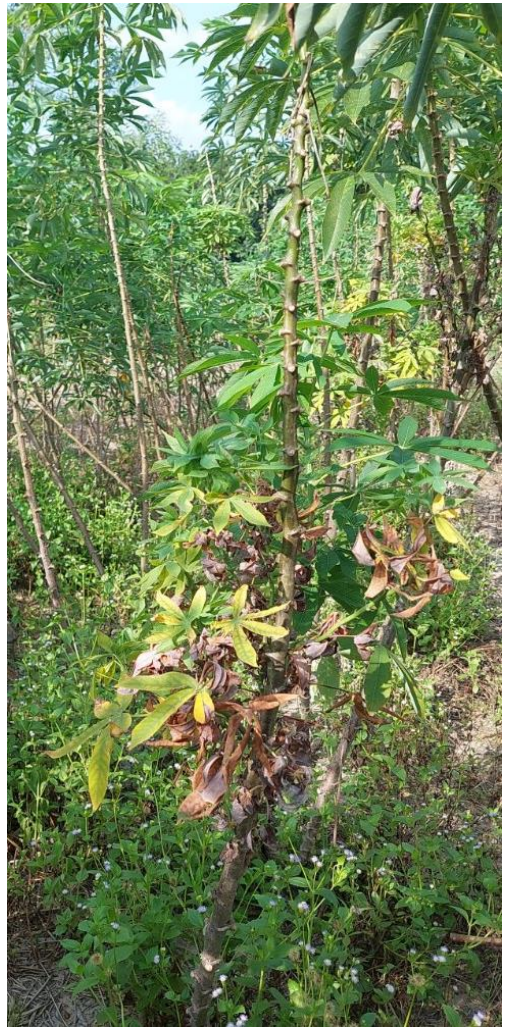
CeratoF2/R2

BJF2/R2

Surveillance activities

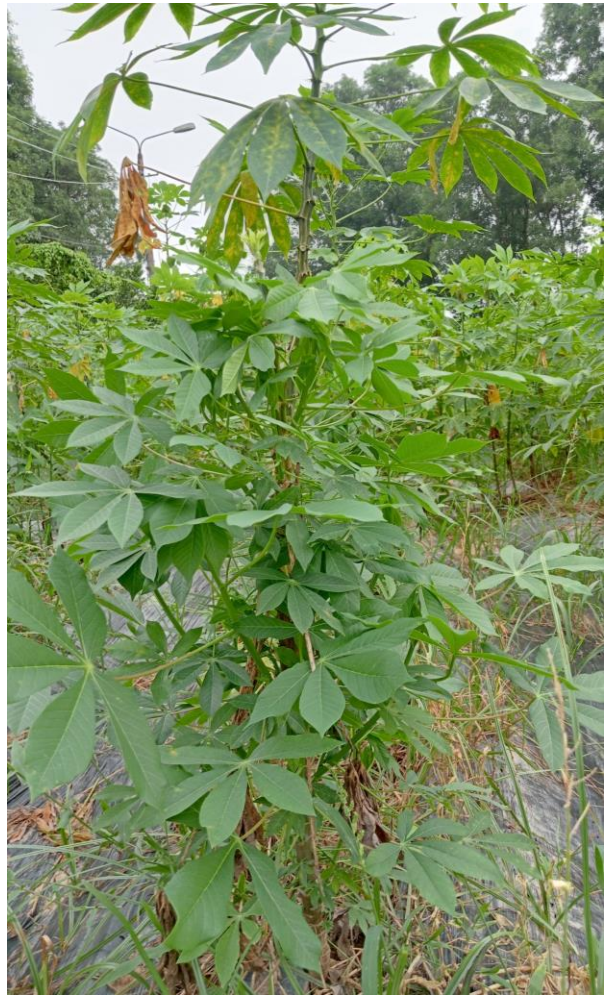
CWBD symptoms



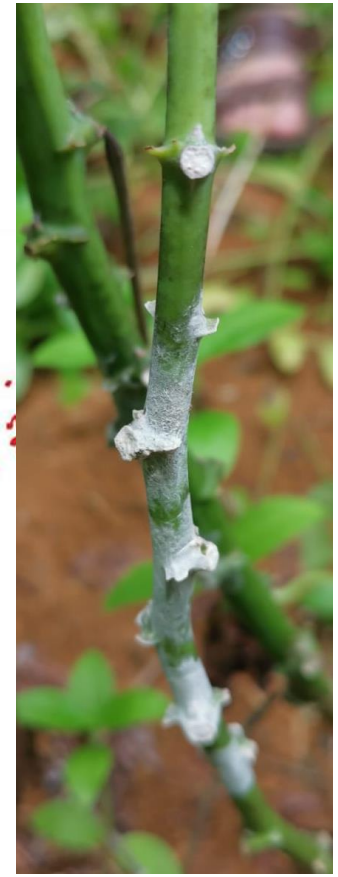



Surveillance activities

Symptom confusion

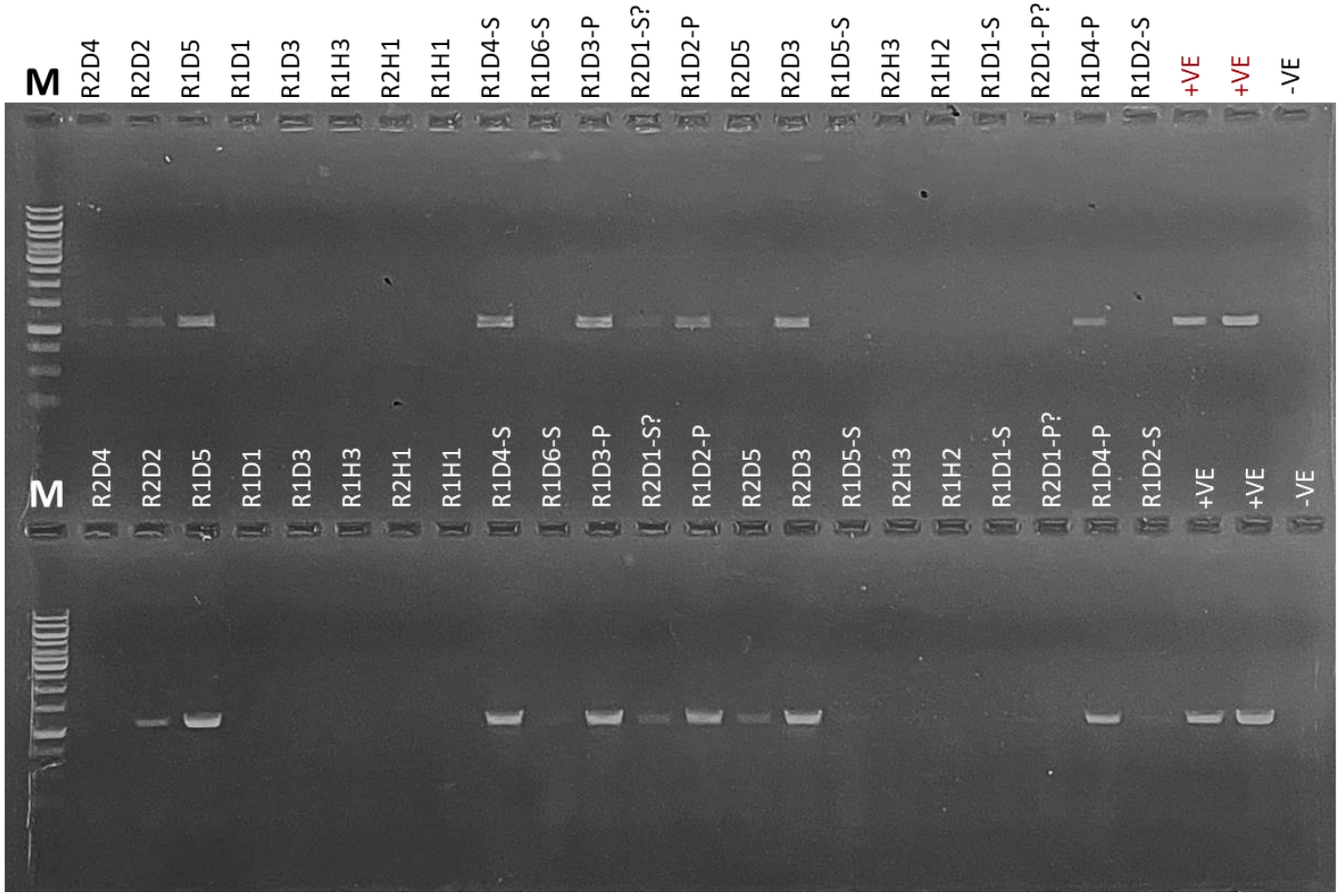


Expanding the scope of disease survey to northern provinces



Expanding the scope of disease survey to northern provinces

Expanding the scope of disease survey to northern provinces



Hanglee.ppri@gmail.com