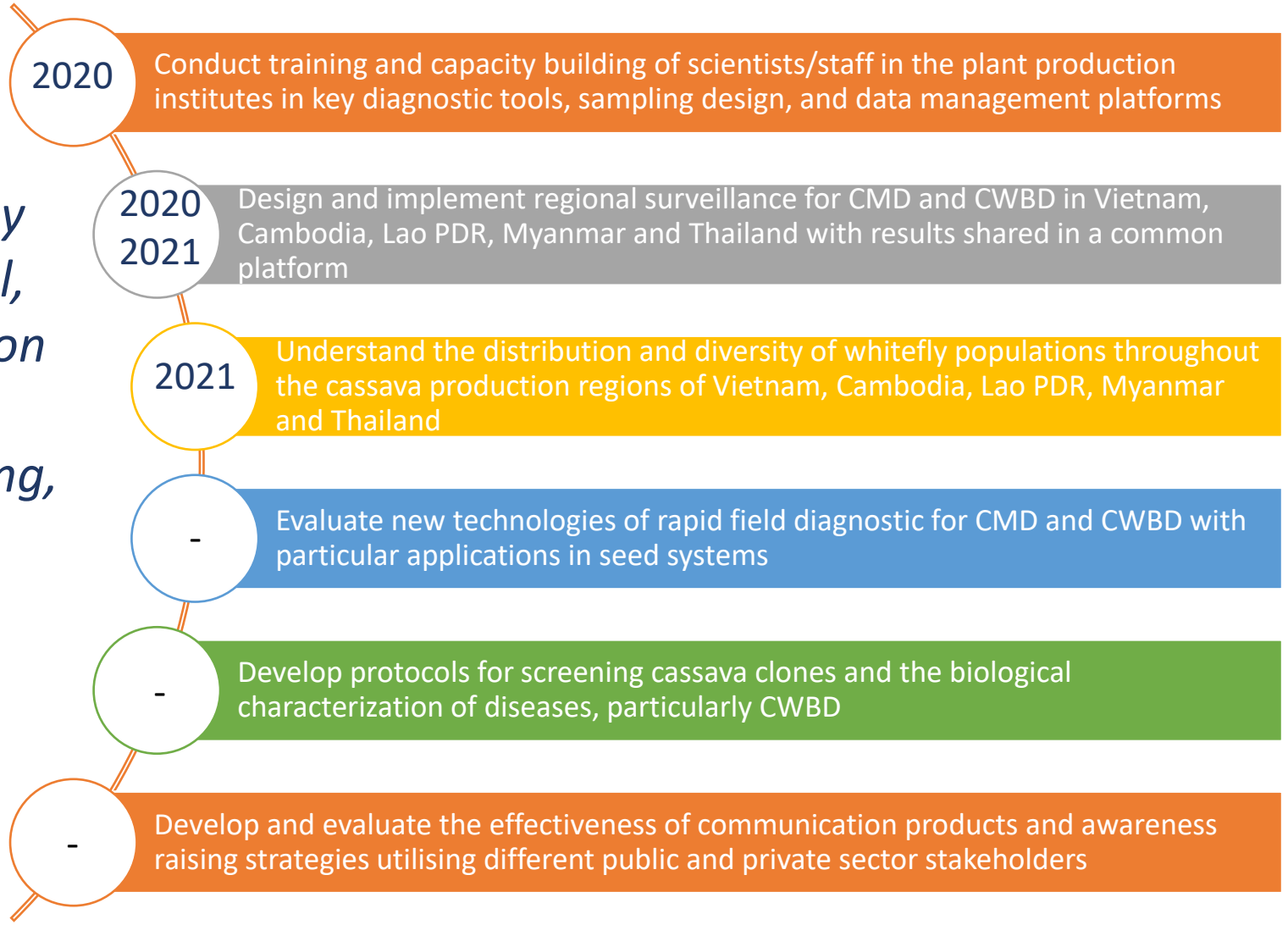
Establishing sustainable solutions to cassava disease in mainland Southeast Asia

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*

Objective 3

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



Summary of achievements in 2020

ID	Activity	Method - Deliverable explanation	Deliverable	Expected Deliverable Date	Date of implimentation	Progress Report on Deliverables (Narrative)
3.1	Conduct training and capacity building of scientists/staff in the plant protection institutes in key diagnostic tools, sampling design, and data management platforms.	• Inventory of existing diagnostic and surveillance protocols. Regional workshop to agree on BSP. • Update and validate protocols with new diagnostics technologies as they are developed.	Attend training and capacity building of plant protection institutes in key diagnostic tools, sampling design, and data management platforms - report	Apr-20	Feb-20	1. Attended online meetings with partner experts (Dr. Wilmer, Dr. Jono, Maria Isabel…) to discuss diagnostic tools, the protocol and plans of sampling, CIAT's data management flatform PestDisPlace. 2. Set up goals and plans for the implementation of the tasks 3. Training and capacity building of PPRI's staff on laboratory techniques including: - Recognize the symptomatic and asymptomatic plants; - Method for surveying in the field, collecting CMD and whitefly samples; - Test and optimize DNA extraction methods for PCR assays of detection of CMD and CWBD; - PCR assays, LAMP for detection of CMD, CWBD
			Training material developed for use within the region	Apr-20	May-21	Contact to Plant Protection Sub-Department of provinces and famers to collect information on areas and disease situation of cassava growing regions across the country Collection and detection Cassava Witches' Broom Disease for production diagnosis Kit
3.2	Design and Implement regional surveillance for CMD and CWBD in Vietnam, Cambodia, Lao PDR, Myanmar and Thailand with results shared in a common platform.	• Regional training courses for field sampling (identification of sampling zones, field-sampling procedures, etc.) • Regional training courses for laboratory (nucleic acid extraction, DNA amplification and analysis, etc.)	• Protocols for uploading and accessing data.	Sampling in Sep-Nov Yr 2,3,4	Jul-20 to Dec-20	Received the protocol and requires for uploading and accessing data on a common tool - Onedrive since early June. Received the protocol for field sampling.
			• Generate and update maps with "confirmed/suspected/non-infected" data sampling	Sep-Nov Yr 2,3,4		1. Implement surveys for CMD, CWBD and whiteflies in sampling locations in: • Northern Midlands and Mountainous: Complete surveillances for 19 locations of 17 districts in 12 provinces. Total number of surveyed field sampling is 72. • Central and the Southern up to December 31st, 2020: -> Total of 280 sampling fields of 61 locations. => The total of 352 sampling fileds of total 79 locations across Vietnam 2. The storage of leaf samples is carried out in the lab by drying at 60°C/3 hours and then place in Ziplock bag containing fresh Silica gel. 3. Generating the data of samples collected for testing the sampling protocol. Updating the data on the common tool - Onedrive.
3.3	Understad the distribution and diversity of WF populations thoughout the cassava production regions of Vietnam	• Sampling of whiteflies • Storage and direct PCR for individual flies for DNA barcoding • Phylogenetic analysis of mtCOI gene	A first regional indexed collection of cassava wfs . Sequence diversity of WF populations. Online access to SEA WF Distribution maps via PestDisPlace	Jul-21		Whitefiles were collected at all the sampling fields and stored in eppendorf tubes 1.5ml containing Ethanol 96%. Total number of tubes collected are 100.
3.4	Evaluate new technologies for rapid field diagnostics for CMD and CWBD with particular applications in seed systems.	Test complete diagnostic system in cassava growing regions in which the diseases are known to exist. Correlate results with PCR based assays and/or any visual observations of disease.	Report on combine optimized primers, DNA extraction method with portable assay	Jun-20	Sep-20	Carried out experiments in lab including Cassava DNA extraction, detection SLCMV and CWBD of all samples in each field required to checking by PCR (360 samples of 6 fields in the North, 2 in Hoa Binh and 4 in Lao Cai province) PPRI also shared samples (67 tubes of WF and many symptomatic/asymptomatic leaves) to CIAT, so they can run experiments in parallel and do next experiments of the coming activities
			Report on test	Jun-21		All samples that are assymptomatic with CMD and CWBD got negative results

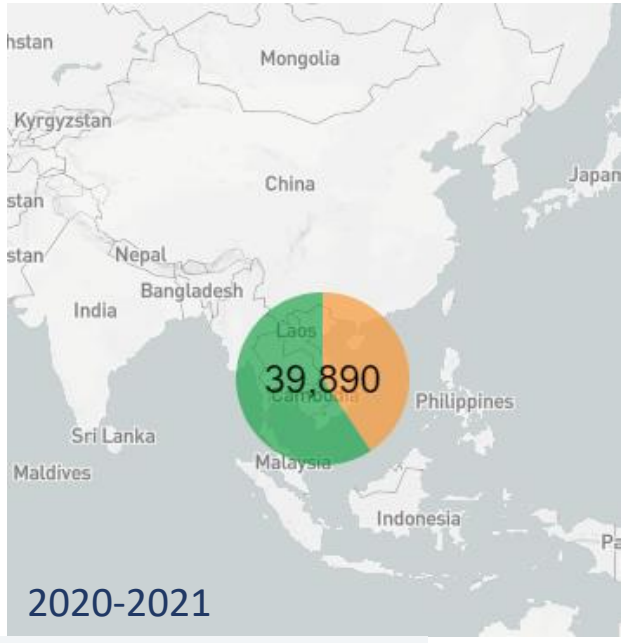
Summary of achievements in 2021

ID	Activity	Method - Deliverable explanation	Deliverable	Expected Deliverable Date	Date of implementation	Progress Report on Deliverables (Narrative)
	Design and Implement regional surveillance for CMD and CWBD in Vietnam, Cambodia, Lao PDR, Myanmar and Thailand with results shared in a common platform.	• Regional training courses for field sampling (identification of sampling zones, field-sampling procedures, etc.) • Regional training courses for laboratory (nucleic acid extraction, DNA amplification and analysis, etc.)	• Protocols for uploading and accessing data.	Sampling in Sep-Nov Yr 2,3,4 Report in June	From 16th Mar To 16th Dec., 2021	Implement surveys for CMD and CWBD in sampling locations as follows:
3.2			• Report	Jun-20	From 16th Mar To 16th Dec., 2021	1. Implement surveys for CMD, CWBD and whiteflies in sampling locations in: - Northern Midlands and Mountainous: Complete surveillances for 18 locations of 17 districts in 12 provinces. Total number of surveyed field sampling is 36 fields - The Central provinces: Complete surveillances for 22 locations of 19 districts in 15 provinces. Total number of surveyed field sampling is 50 fields - The Southern provinces: Complete surveillances for 14 locations of 19 districts of 5 provinces. Total number of surveyed field sampling is 42 fields. The storage of leaf samples is carried out in a Ziplock bag containing Silica gel (change fresh Silica gel every 2 weeks or until discoloration of silica gel is observed)"
3.3	Understand the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam, Cambodia, Lao PDR, Myanmar and Thailand.	• Sampling of whiteflies designed during activity 2.1 • Storage and direct PCR for individual flies for DNA barcoding • Phylogenetic analysis of mtCOI gene to sequence-characterize to identify the different populations	A first regional indexed collection of cassava WF Sequence diversity of WF populations in SEA identified and characterized Online access to SEA Whitefly Distribution maps via PestDisPlace	Jul-21		Sampling of whitefiles in locations as disease surveys. 80 tubes from 94 files were collected in total (stored in Eppendorf tubes 1.5ml containing Ethanol 96°). Take photos of the 5 youngest leaves of 30 plants => Evaluate the correlation between the cassava leaves and whiteflies in Vietnam by determining the density of whiteflies on the first 5 youngest leaves 88 samples of whiteflies collected during 2020-2021 surveys throughout Vietnam have been sent for sequencing. Once the results are sent in, the phylogenetic analysis of mtCOI will be performed.
3.4	Evaluate new technologies for rapid field diagnostics for CMD and CWBD with particular applications in seed systems.	• Design and test LAMP primers for SLCMV and CWB • Combine optimized primers, DNA extraction method with the portable assay device to create an entire diagnostic system	Report on combine optimized primers, DNA extraction method with portable assay	Jun-21	Jan-May, 2021	Focus on new technologies for rapid filed diagnostics for CMD and CWBD, and also seed systems: - Develop DNA extraction methods for detection of viral diseases of Cassava cutting stems: DNA extraction from phloem tissues, which is supposed to contain the virus, is very important in the selection of disease-free varieties for a new crop. - Access to technology of production of polyclonal antibodies as tools for detection of CMD.
3.4		• Test complete diagnostic system in cassava growing regions in which the diseases are known to exist. Correlate results with PCR based assays and/or any visual observations of disease.	Report on test	Jun-21		

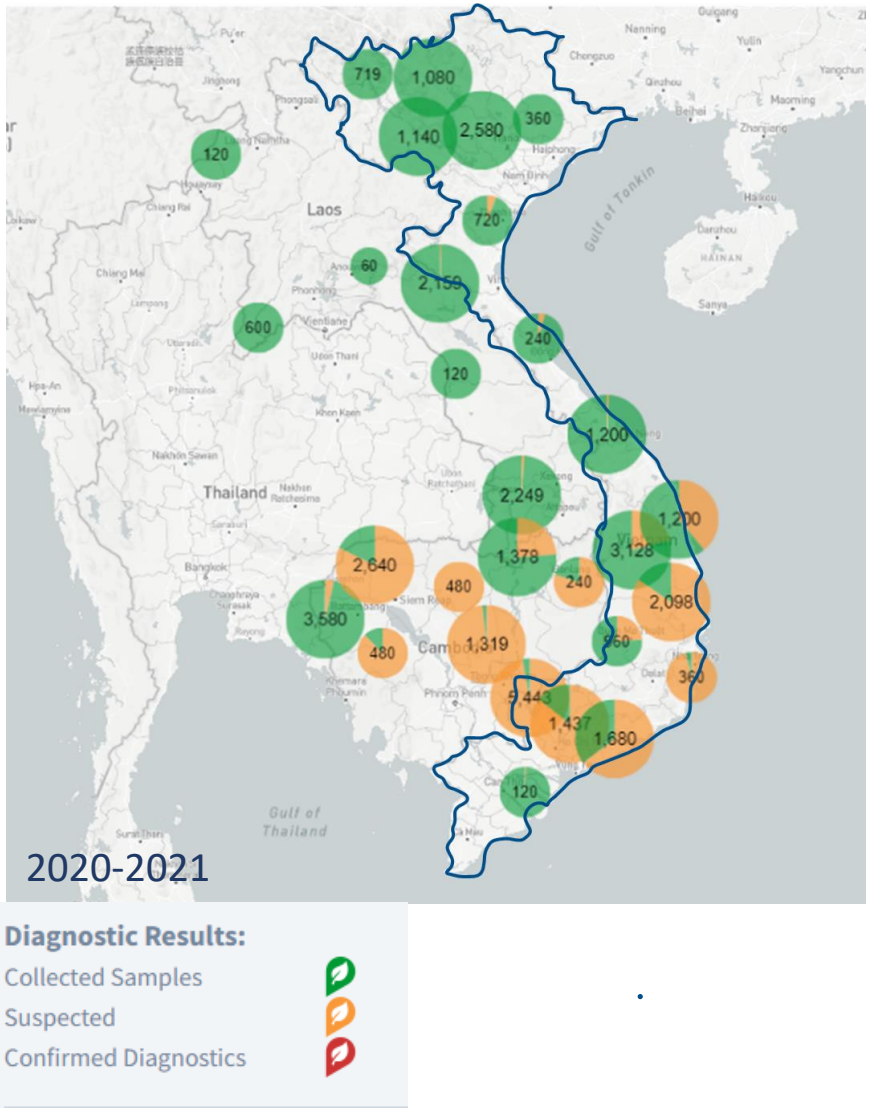
Surveillance

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

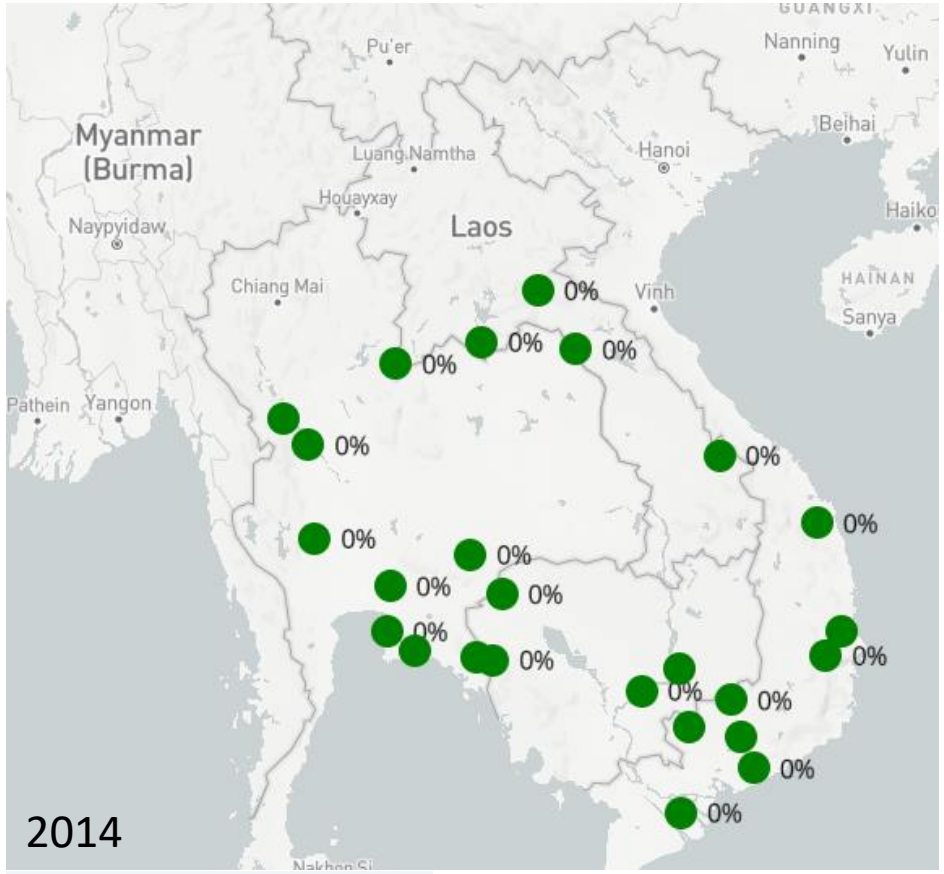
- ✓ Conducted surveys and sampling on **479 fields**
- ✓ All data is shared on a common platform
- ✓ **39,890** observations were shared on PestDisPlace that conducted by the 3 country teams during the two consecutive years 2020-2021
- ➢ **24,345** observations in Vietnam alone



24,345 observations



Incidence Maps of CMD

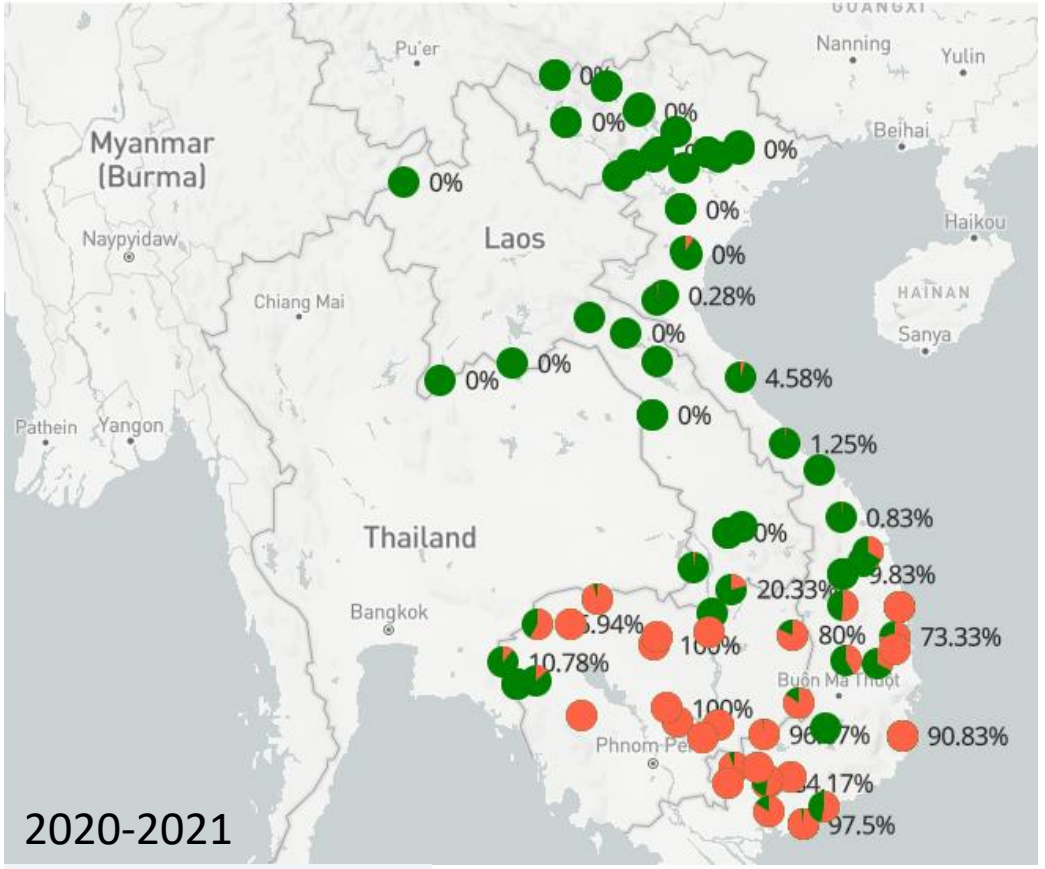


Diagnostic Results:

Collected Samples

Suspected

Confirmed Diagnostics



Diagnostic Results:

Collected Samples

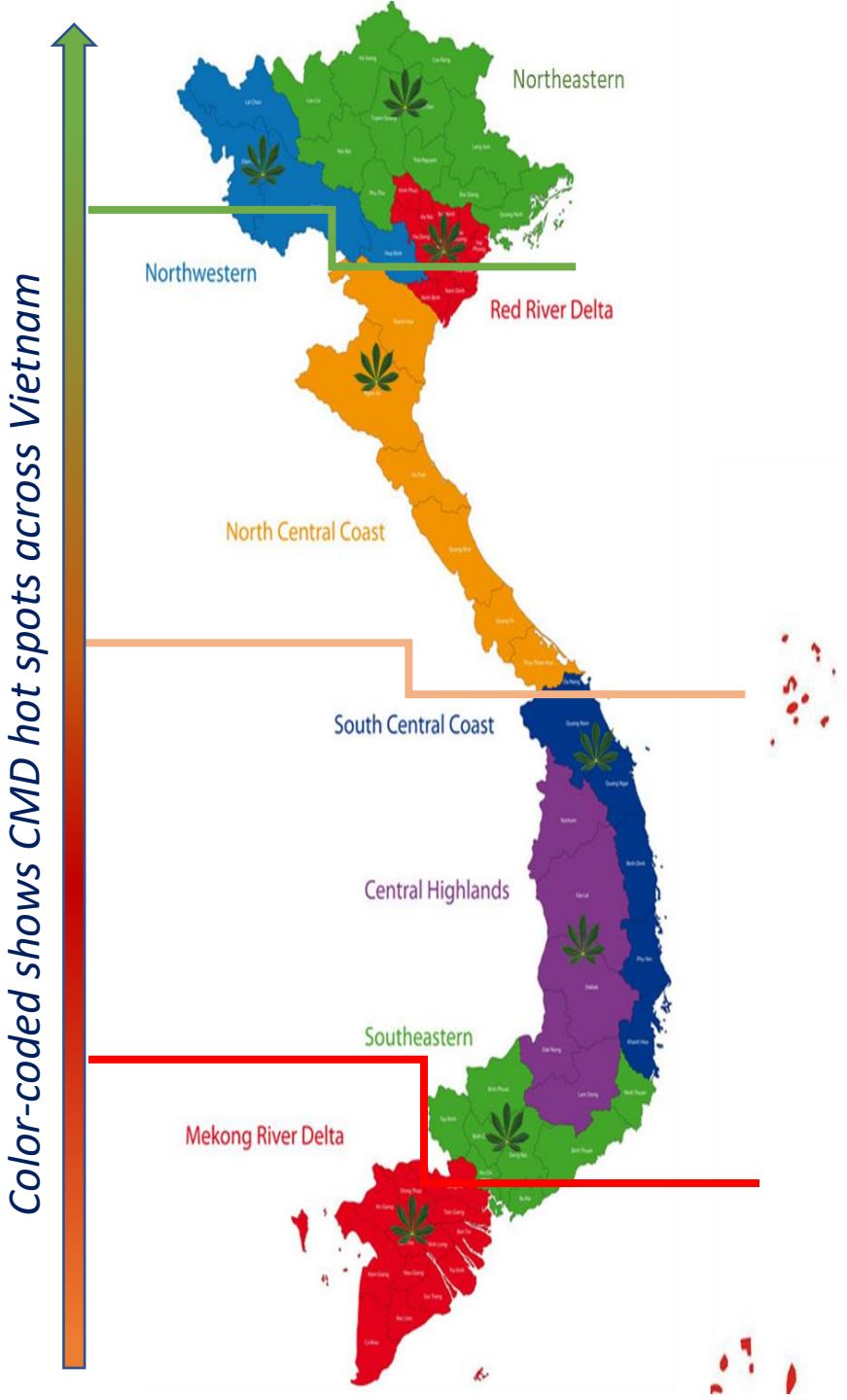
Suspected

Confirmed Diagnostics

Surveillance

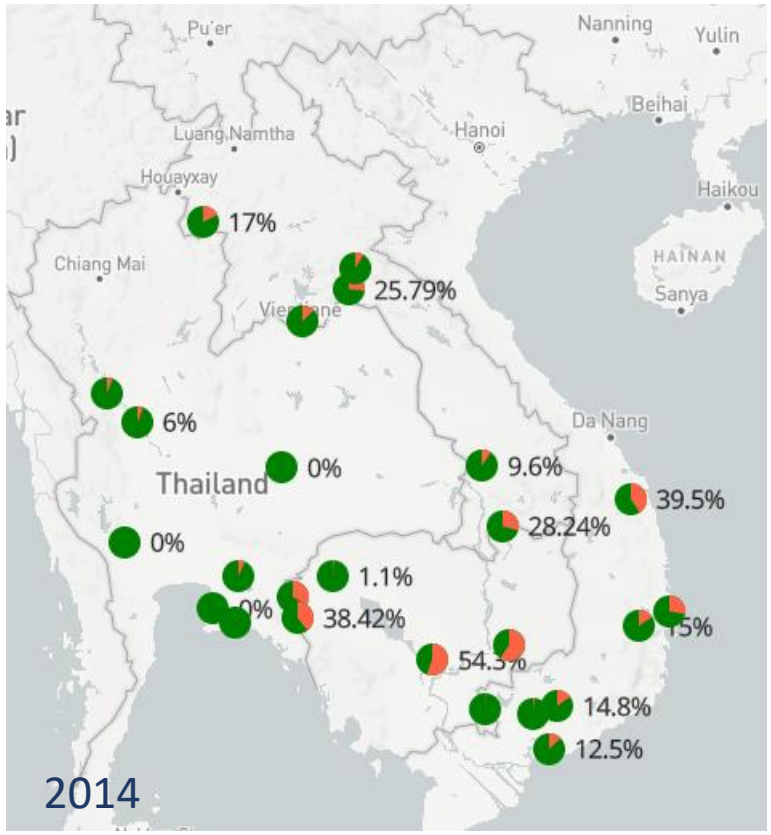
CASSAVA MOSAIC DISEASE (CMD)

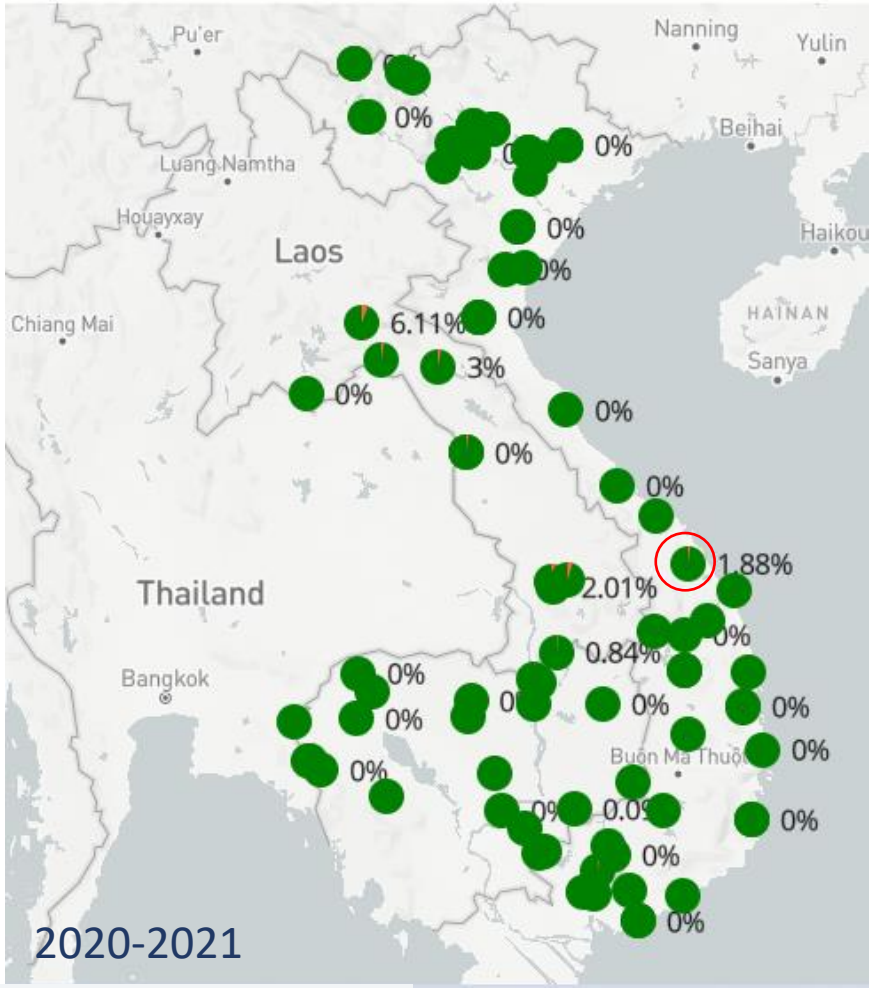
- First reported since 2016 in Vietnam
- CMD has emerged as one of the most serious threats to cassava production
- The quick spread and serious damage of CMD from ***South to North***
- *Sri Lankan cassava mosaic virus* (SLCMV) is the causal agent of CMD in Vietnam



Incidence Maps of CWBD

- First recorded of CWBD since 2008 in Vietnam
- The quick spread and serious damage of CWBD from 2010-2015
- Almost disappeared from 2015 to present





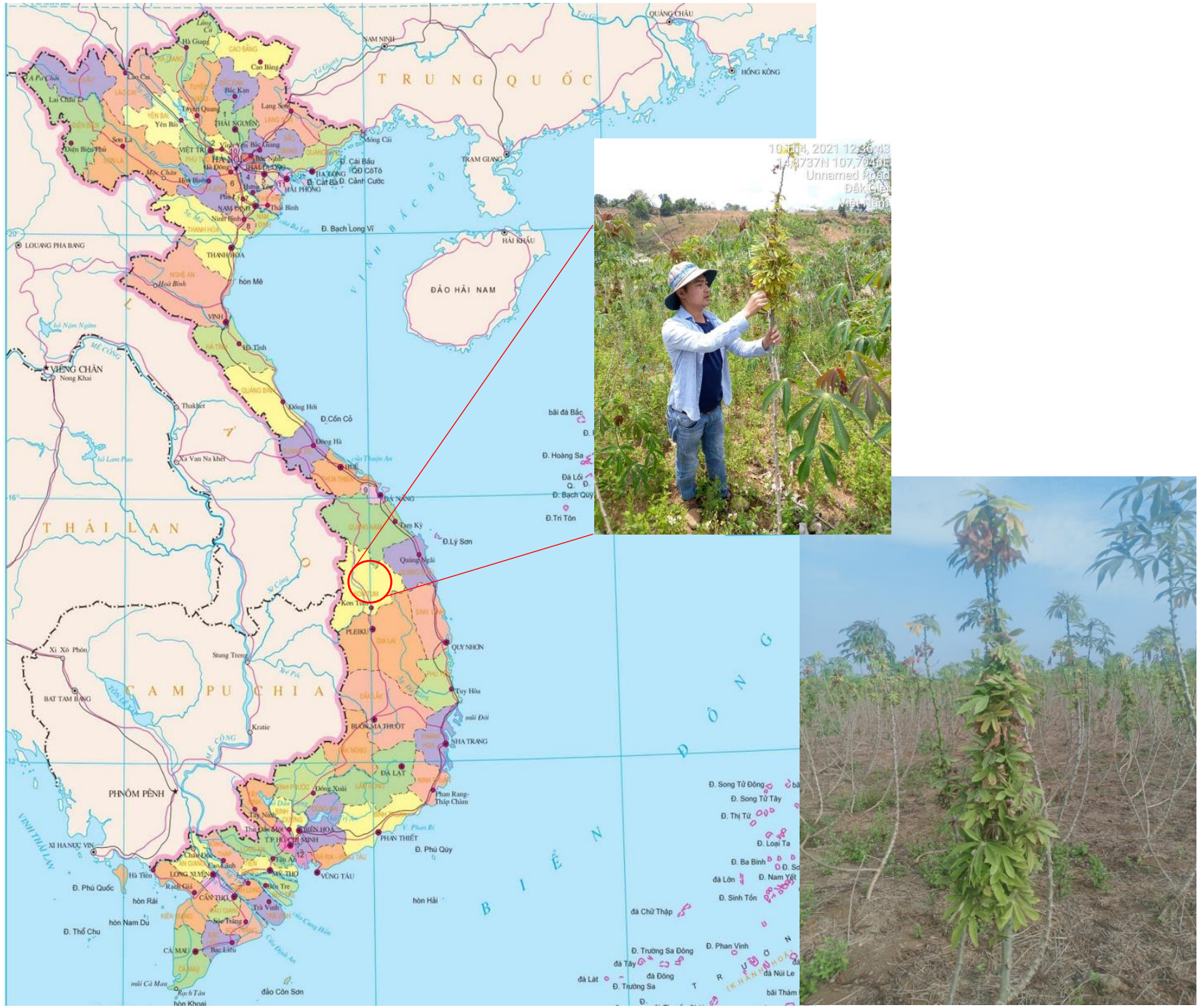
Diagnostic Results:
Collected Samples
Suspected
Confirmed Diagnostics

Diagnostic Results:
Collected Samples
Suspected
Confirmed Diagnostics

Surveillance

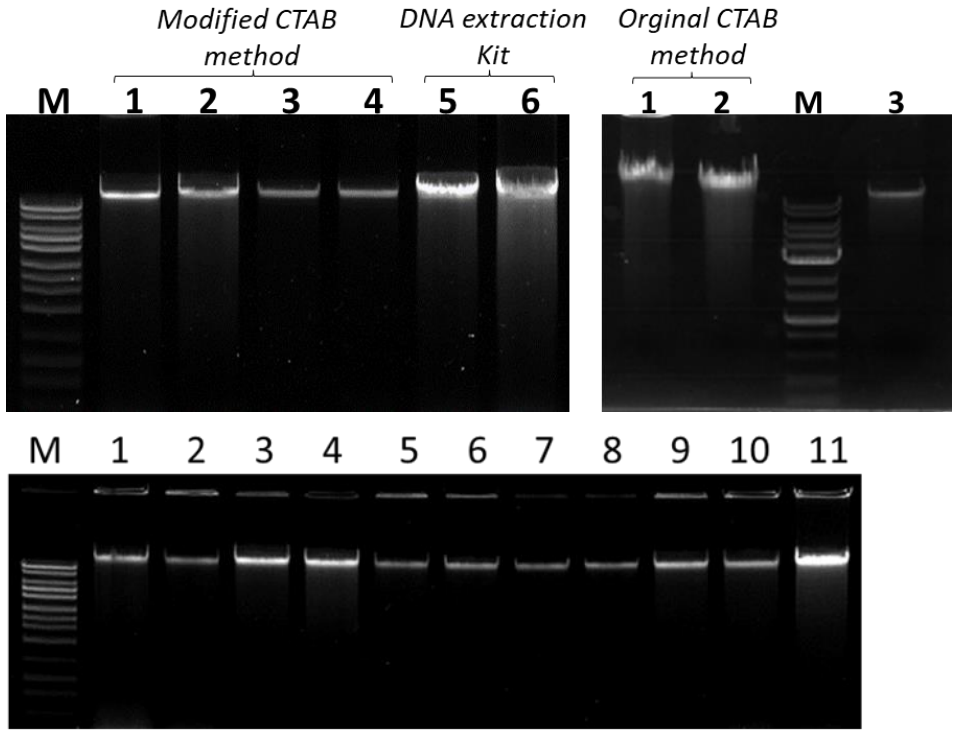
CASSAVA WITCHES' -BROOM DISEASE (CWBD)

Field surveillance was carried out in late April 2021 in Kontum province. The result has notably shown that CWBD was coming back after a long absence (test by Nested PCR)



Diagnostic

Rapid and efficient isolation of high quality DNA from Cassva leaves suitable for PCR test since pooled sample testing is applied for large-scale detection



M: HighRangger 1 Kb DNA Ladder

1-8: *Dried samples* (healthy young leaves were collected in the field, placed in a bag containing silica gel before being dried at 60°C/3hrs and keeping at room temperature.

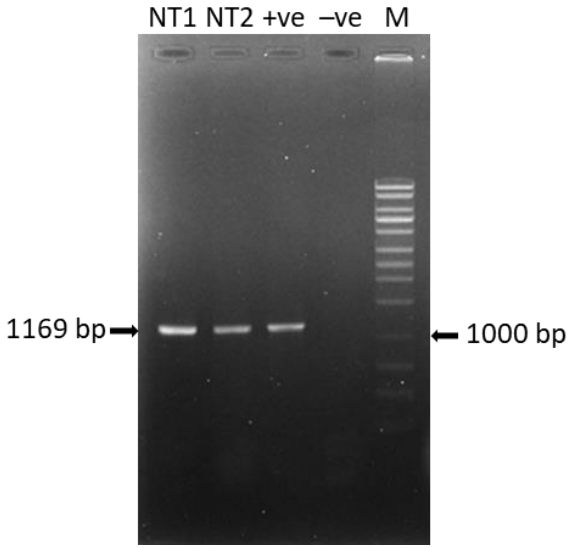
9-10: *Fresh samples* (healthy young leaves in PPRI greenhouse).

11: *Frozen sample* (healthy young leaves were collected in the field, stored at -80°C until use

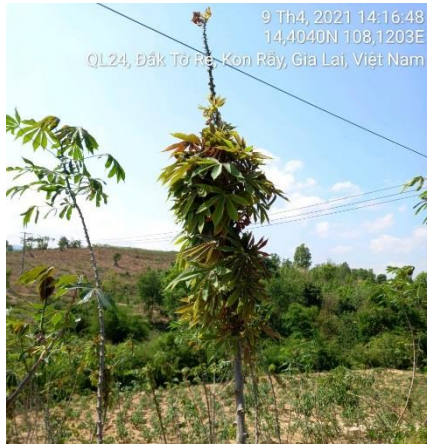
No.	Sample ID	DNA Concentration (ng/µl)	A_{260}/A_{280}	Volume/ lane (µl)
1	Marker			6
2	1	3264.7	1.96	8
3	2	413.6	1.88	8
4	3	3234.7	1.93	8
5	4	2541.7	1.87	8
6	5	1028	1.77	8
7	6	1418.8	1.77	8
8	7	1860.5	1.78	8
9	8	2653.8	1.88	8
10	9	2331.3	1.92	8
11	10	2573.3	1.85	8
12	11	3196.2	1.95	8

Diagnostic

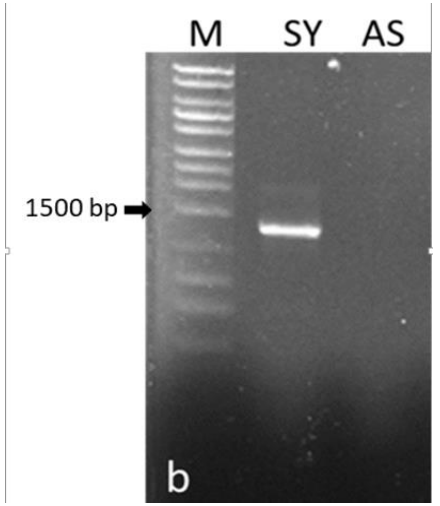
Extraction of genomic DNA from Cassava leaf using modified CTAB method



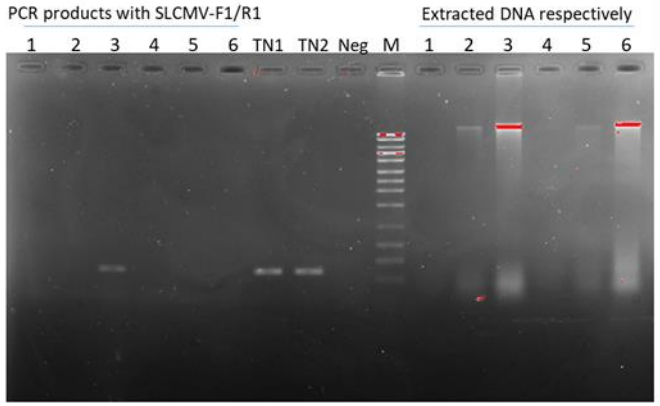
Detection of SLCMV by PCR



P1/P7-
R16F2n/R16R2



Detection of CWBD by nested PCR



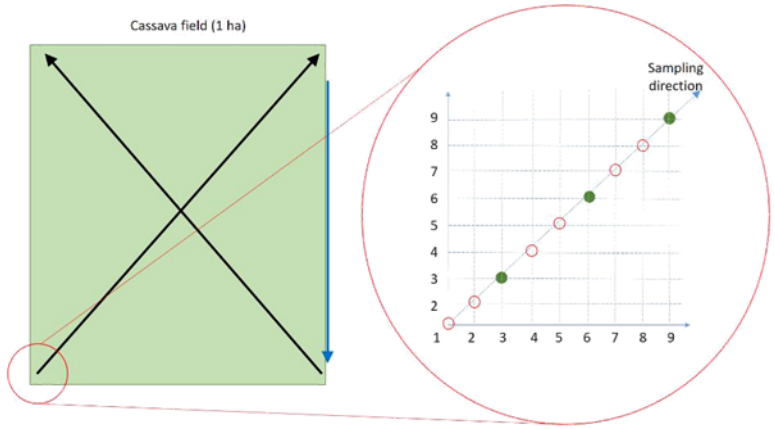
1, 2, 3: old stem section (1-Phloem, 2-Bark, 3-Bud)
4, 5, 6: young stem section (4-Phloem, 5-Bark, 6-Bud)

Develop DNA extraction methods for detection of viral diseases of Cassava cutting stems

Surveillance of Whitefly

✓ **Sampling of whiteflies designed during surveys**

The surveillance of whiteflies followed by the protocol of CIAT

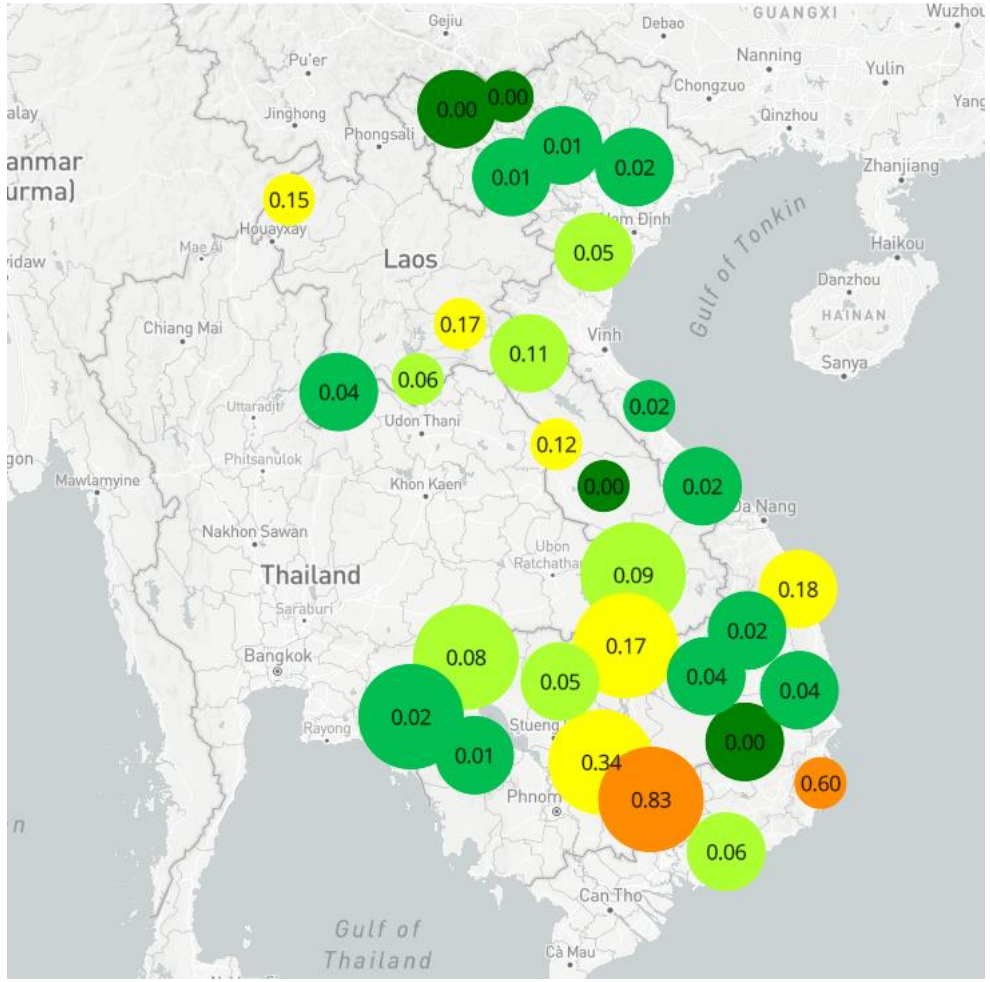


30 plants were evaluated following a diagonal in each fields

The evaluation was made evaluating photographs of the second youngest leaf

Surveillance of Whitefly

- ✓ Sampling of whiteflies designed during surveys
- ✓ Photo data were shared on a common platform

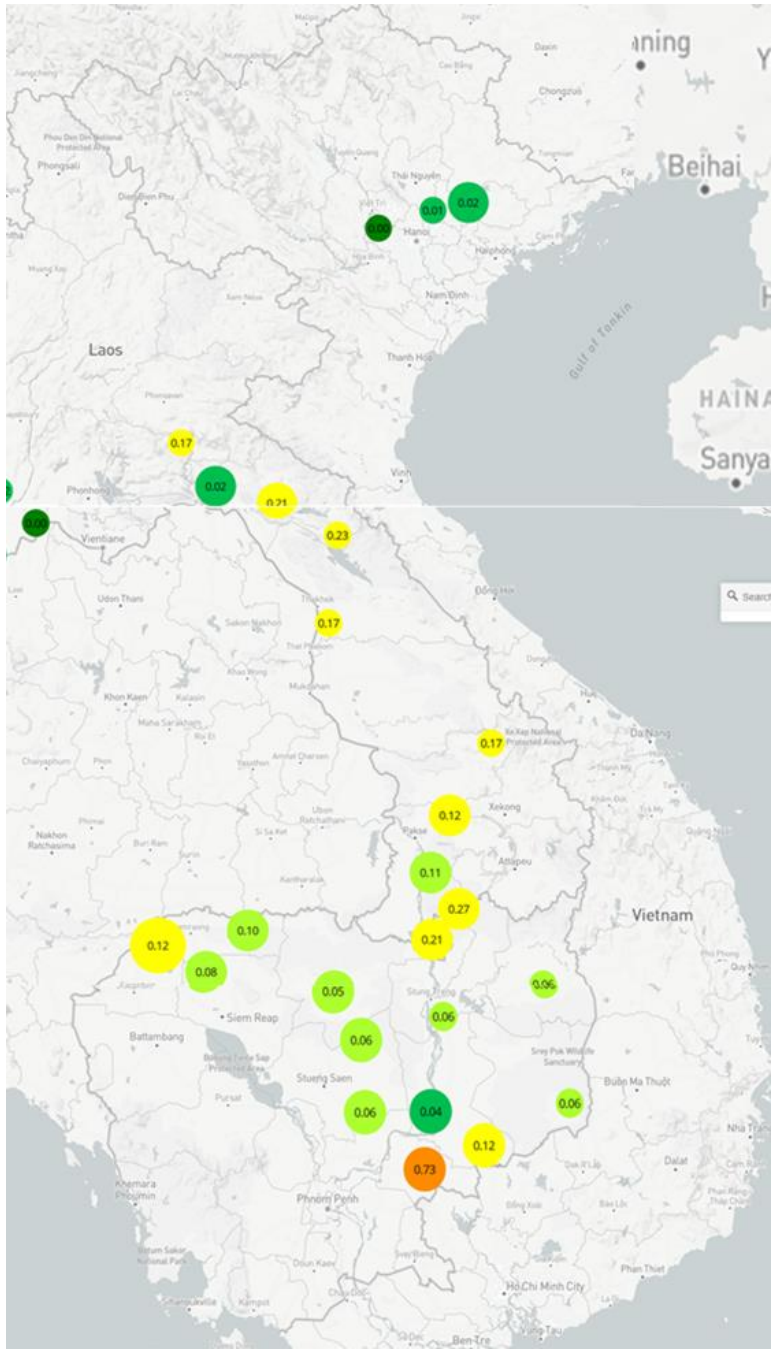


Range		Frequency
0		394
>0	<=0.04	116
>0.04	<=0.11	106
>0.11	<=0.5	112
>0.5	<=1.1	16
>1.1	<=20	6

WF relative abundance

2020

WF - 2020

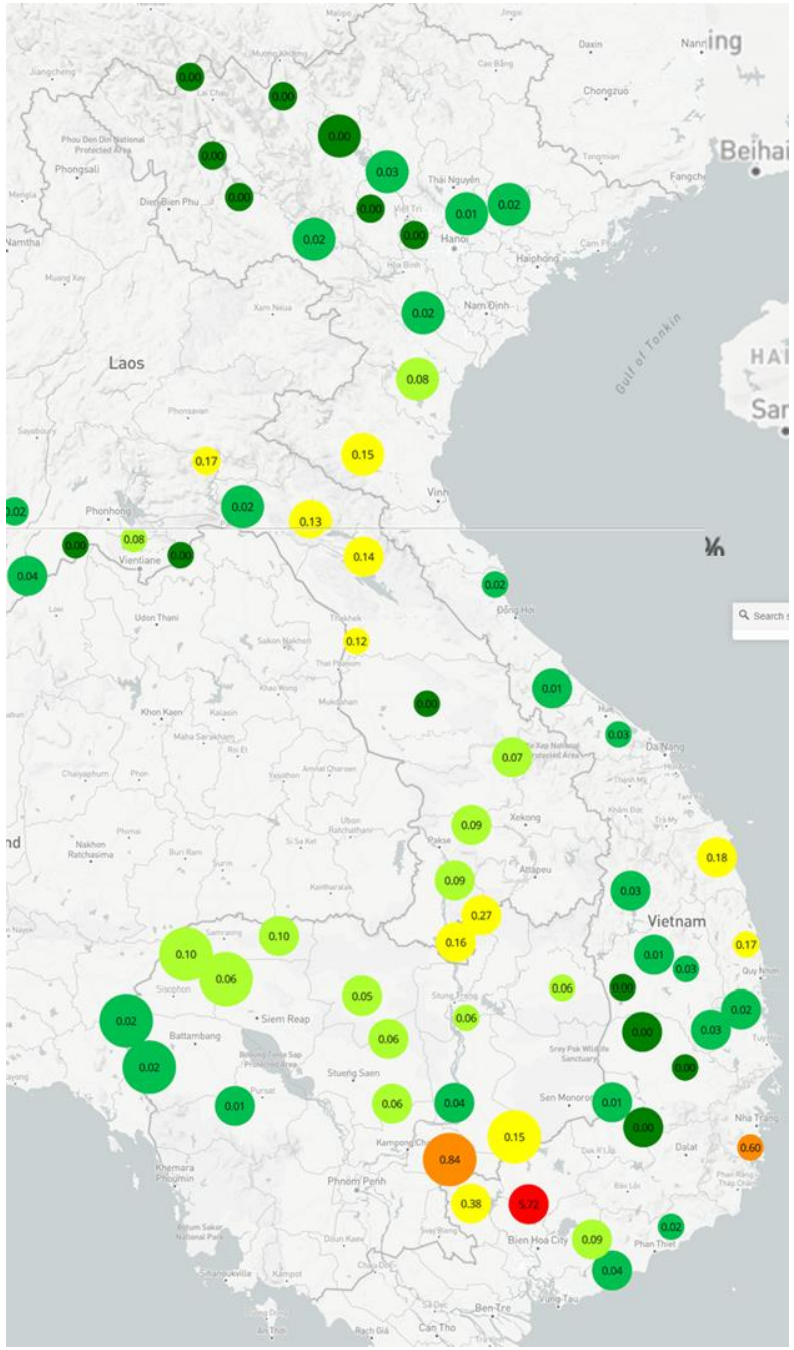


CMD - 2020

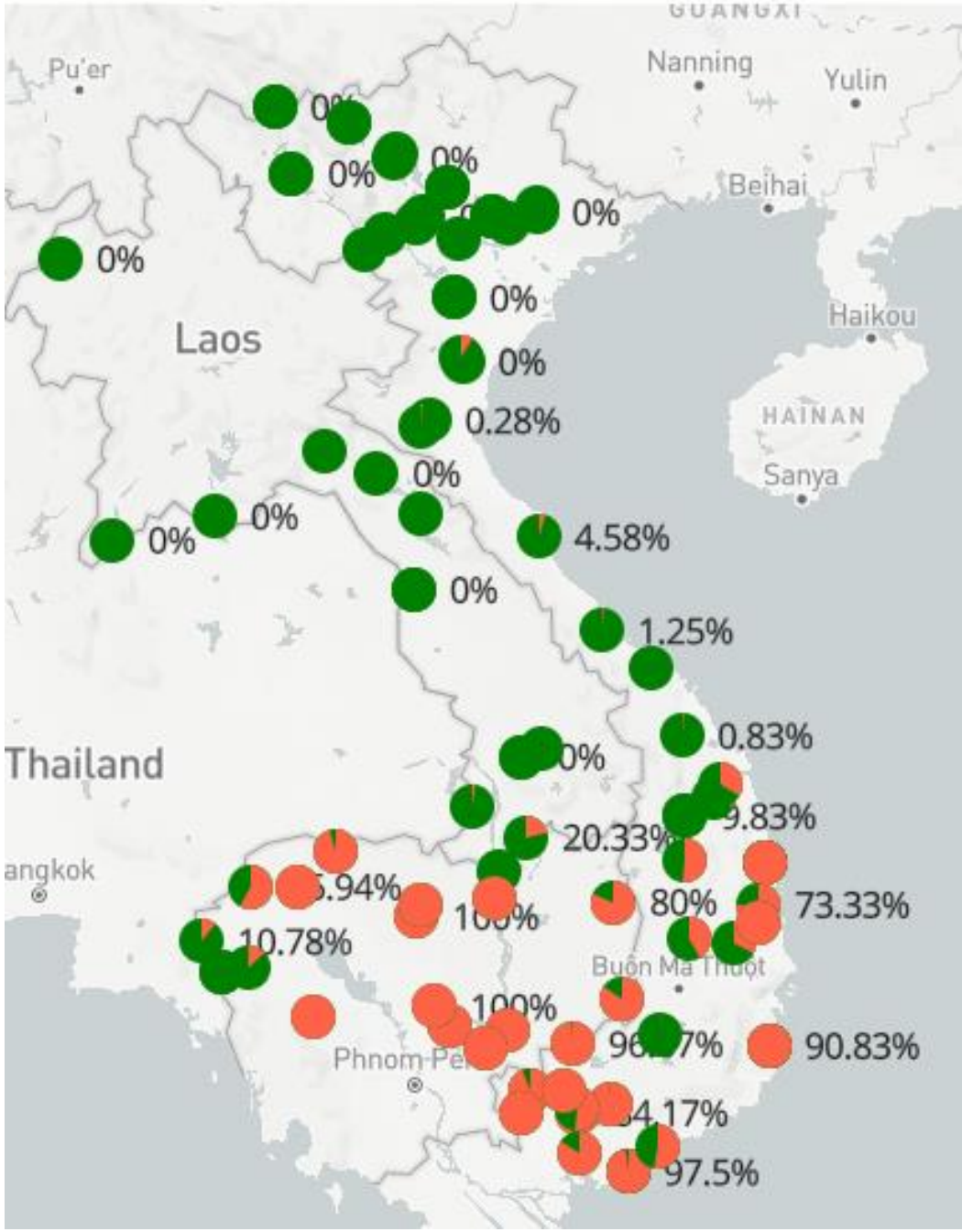
WF relative abundance

2021

WF - 2021



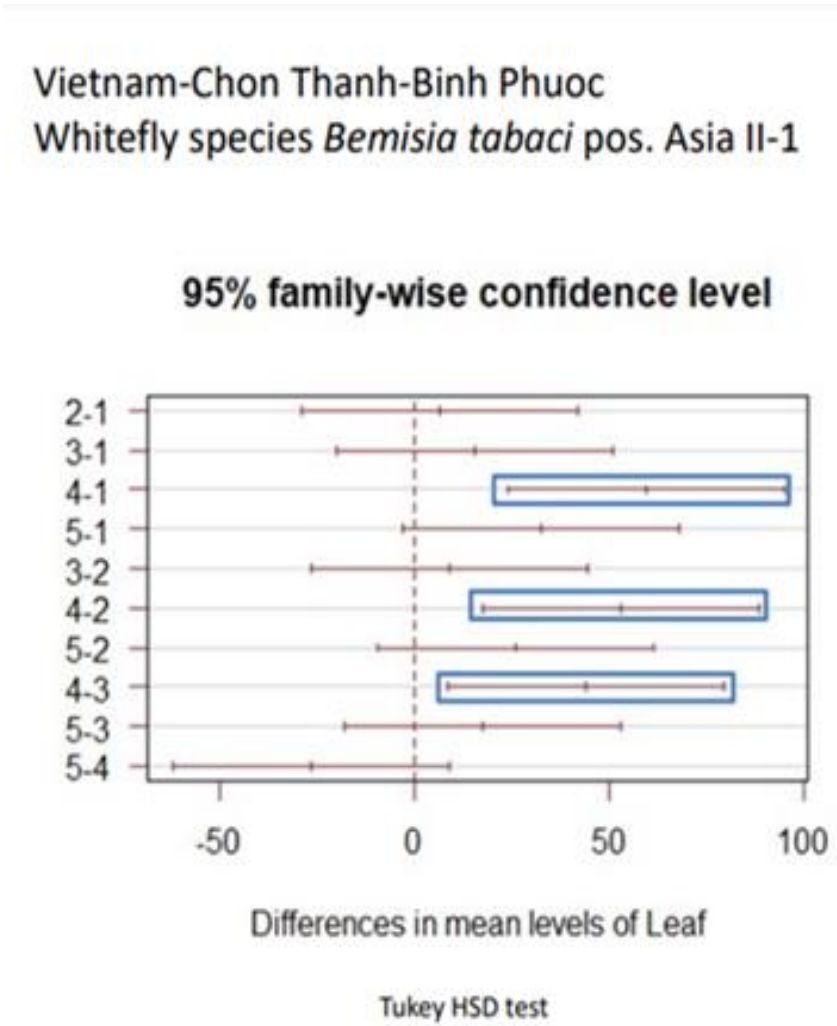
CMD - 2021

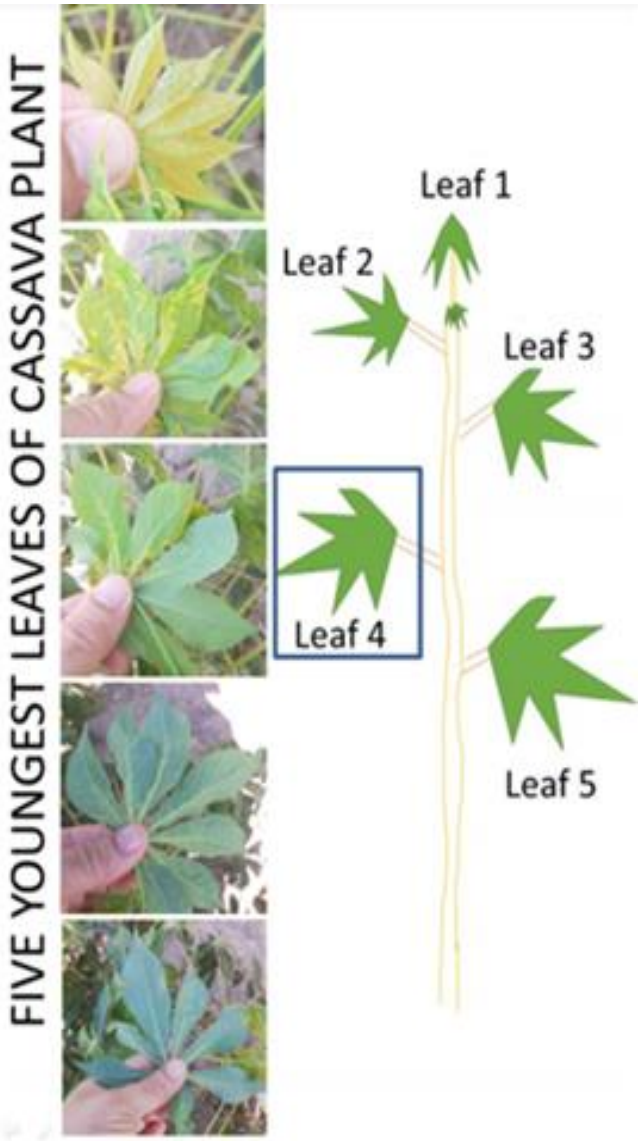


Surveillance of Whitefly

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

=> The preference of WF is the fourth leaf

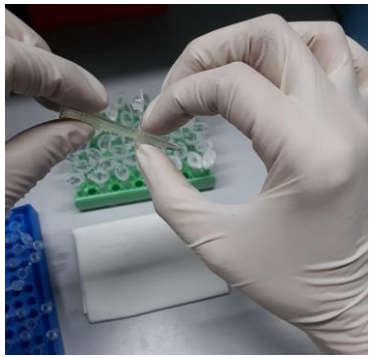


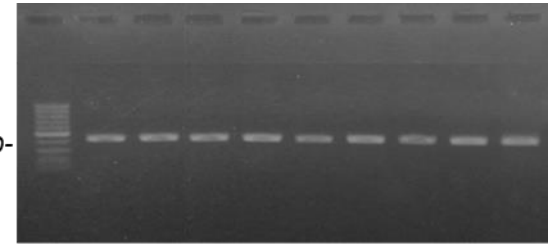


Diversity of Whitefly in Vietnam

Sequencing of 86 whitefly samples collected during 2020-2021 surveys for phylogenetic analysis based on mtCOI gene to sequence characterize the identify and different populations.

Diversity of Whitefly in Vietnam





Whitefly total DNA extraction

- Introduce one whiteflies into a 0.2 mL eppendorf tube and put it into liquid nitrogen or -80°C
- Macerate samples by sterilized pestle to get a fine powder
- Add 4 µL NaOH 50 mM into the tube -> keep grinding
- Rinse the sterilized pestle by 4 µL NaOH 50 mM -> transfer the solution into the first tube -> incubate 5 min at 95°C
- Add 1 volume (8 µL) of Tris-HCl 1M (pH 8.0) into the tube and use the solution for next steps

PCR

Component	Volumes
GoTaq Master Mix	25
Forward Promer	1
Reverse Primer	1
DNA templete	2
Distilled Water	Up to 50 µl

Primer	Sequence (5' – 3')	PCR product
2195Bt	TGRTTTTTTGGTCATCCRGAAGT	850 bp
COI/Bt-sh2	TTTACTGCACTTTCTGCC	

94°C	94°C	55°C	72°C	10°C
2 min	30 sec	30 sec	1 min	∞

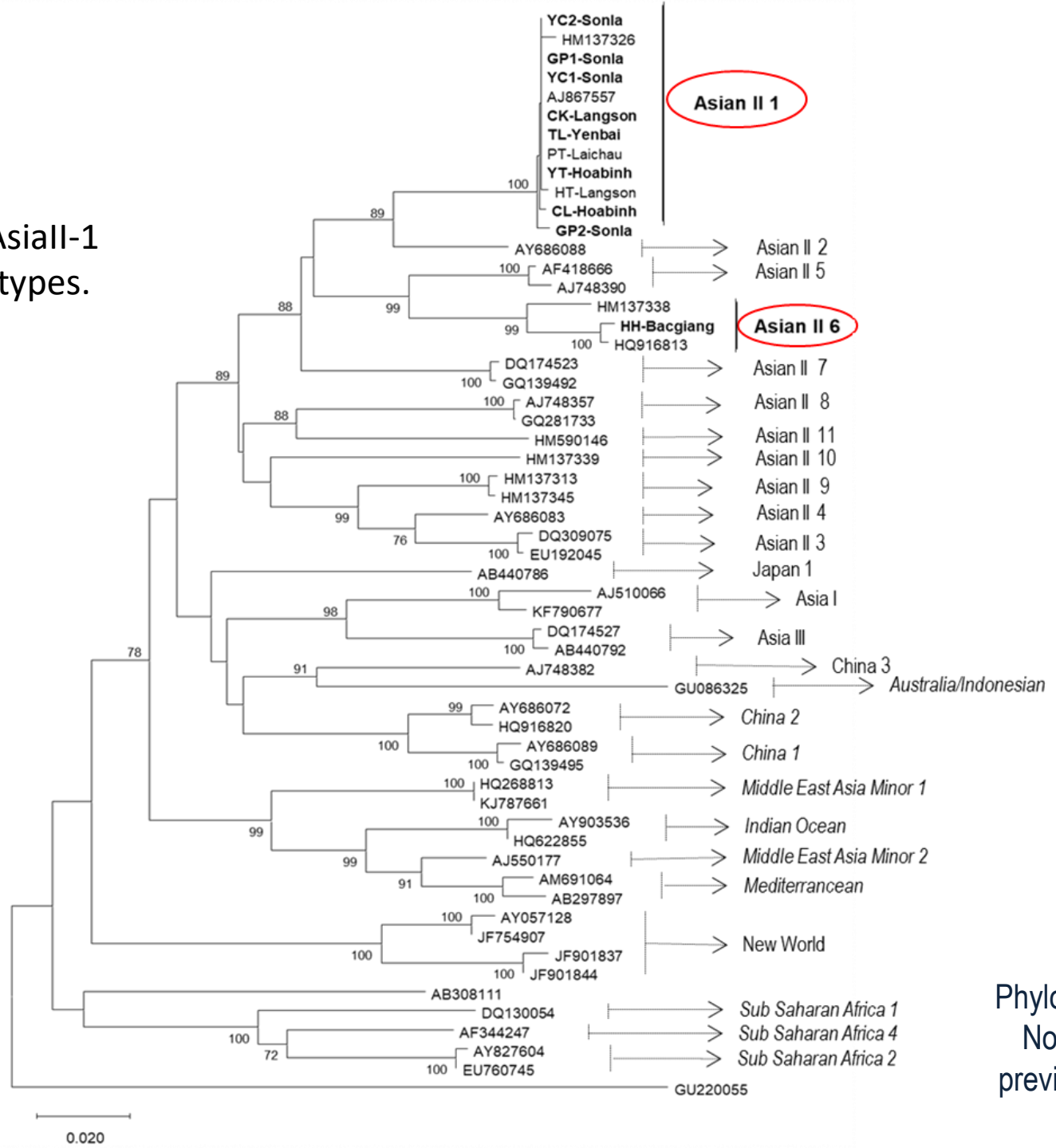
25 cycles

Electrophoresis

Check the PCR product by using 1% agarose gel

North Zone

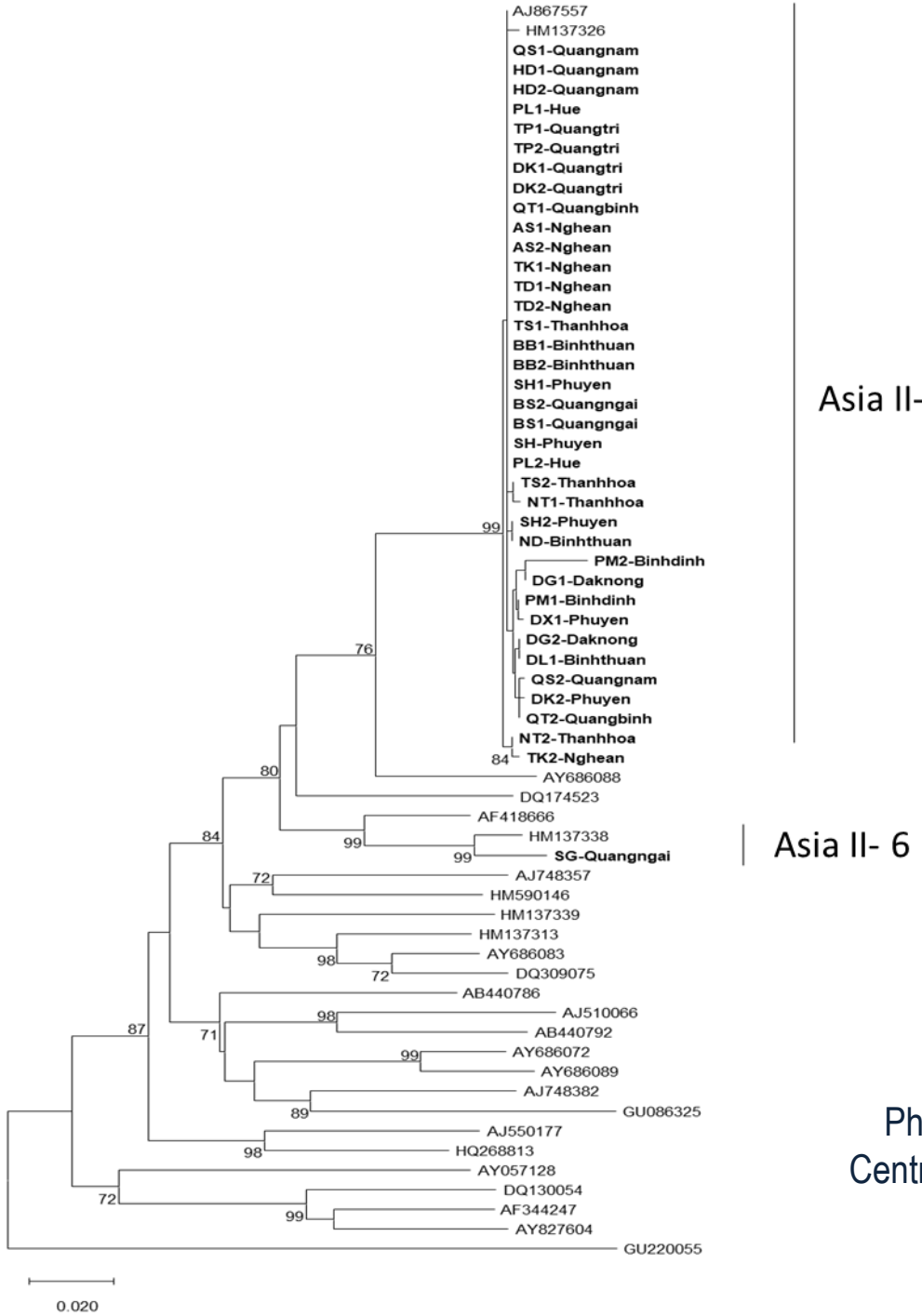
Only detected AsiaII-1 and AsiaII-6 biotypes.



Phylogenetic analysis of *B. tabaci* collected from the Northern provinces along with the sequences of previously identified species/biotypes obtained from database/CSIRO data collection

Middle Zone

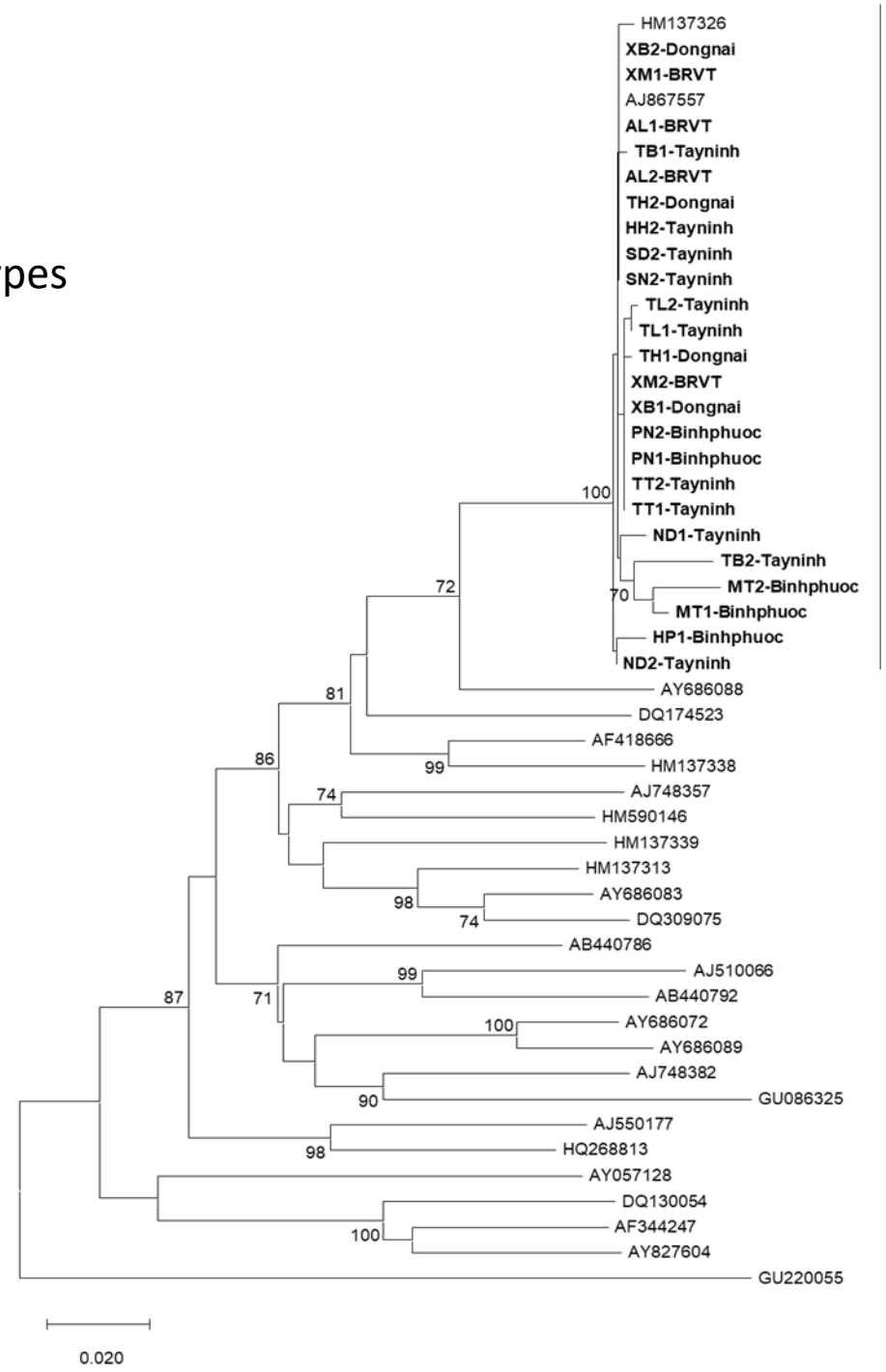
Only detected AsiaII-1 and AsiaII-6 biotypes



Phylogenetic analysis of *B. tabaci* collected from the Central provinces along with the sequences of previously identified species/biotypes obtained from database/CSIRO data collection

South Zone

Only detected AsiaII-1 biotypes



Asia II- 1

Phylogenetic analysis of *B. tabaci* collected from the Southern provinces along with the sequences of previously identified species/biotypes obtained from database/CSIRO data collection

In summary

- ✓ 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 with results shared in a common platform
- ✓ Understand the distribution and diversity of whitefly populations throughout the cassava production regions of Vietnam

Hanglee.ppri@gmail.com