



Alliance



# Challenges in Phytosanitary Surveillance for the International Distribution of Germplasm: Phytosanitary Evaluation of the Cassava Collection

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## Germplasm Health Laboratory

November 11th, 2020



**Phytosanitary Awareness Week**  
9 to 13 November 2020

**Phytosanitary Safety for Transboundary Pest Prevention**

CGIAR Germplasm Health Webinar series

NOVEMBER  
9 to 13, 2020

Alliance



# GERMPLASM INTERNATIONAL DISTRIBUTIONS



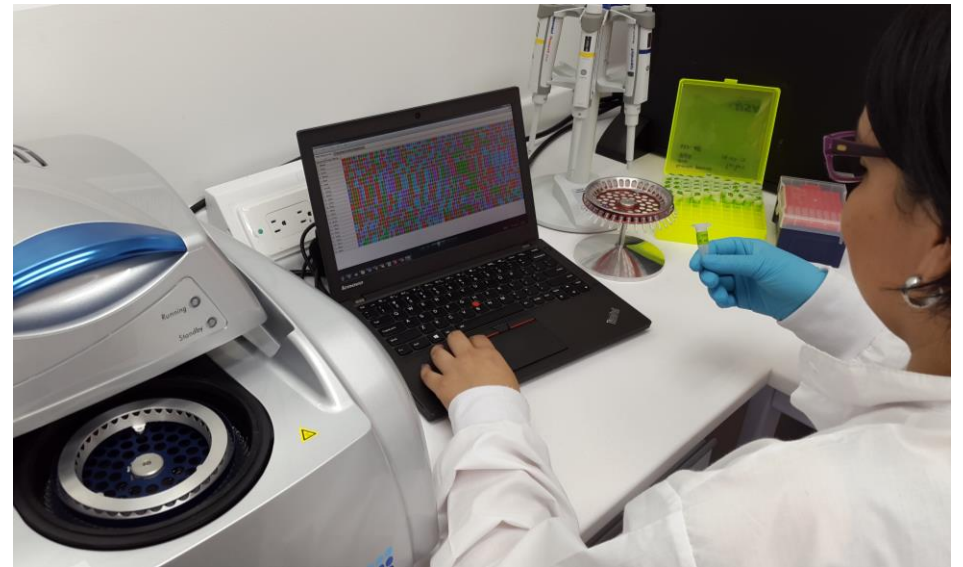
# Germplasm Health Unit

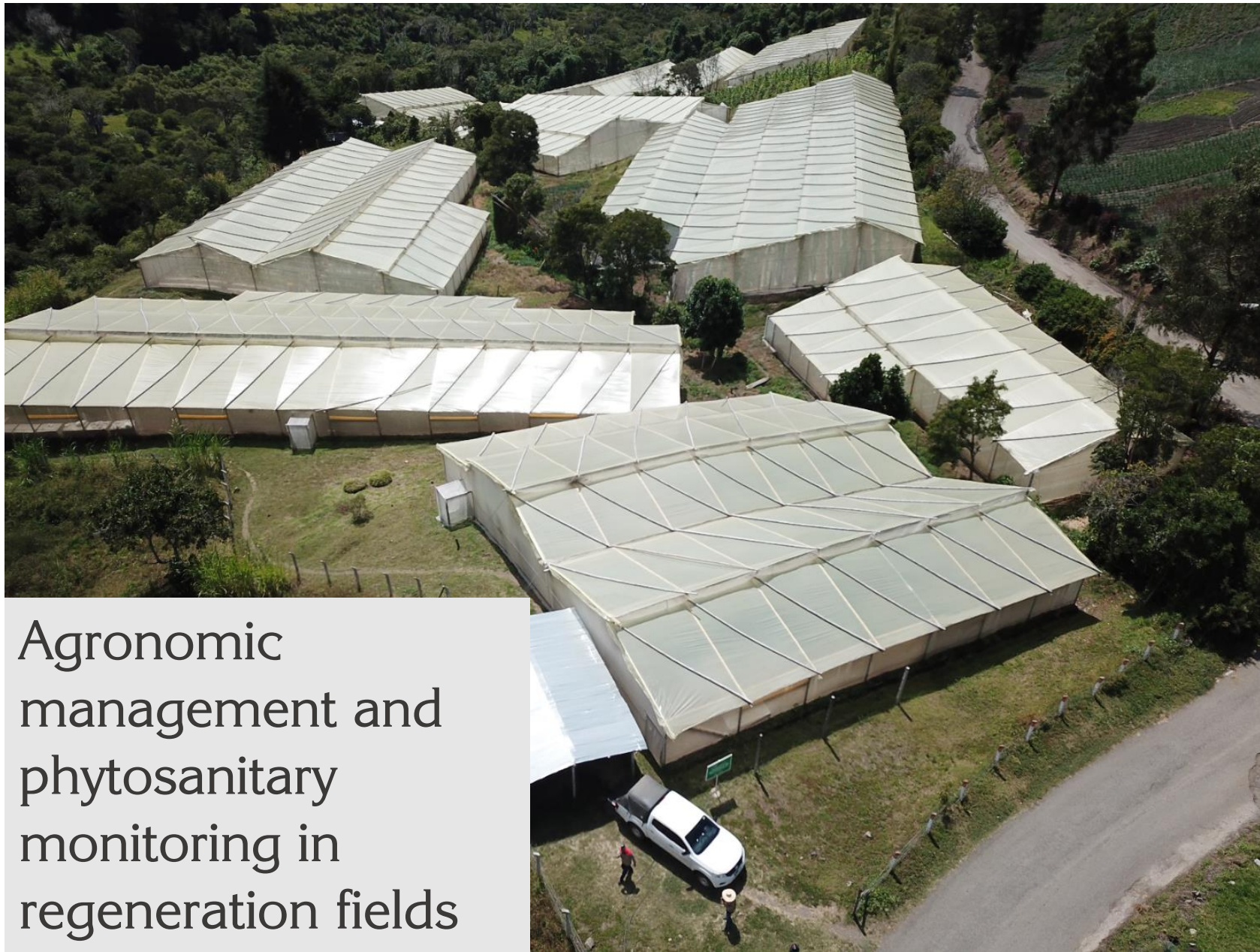
Has the responsibility of evaluating the germplasm distributed by the genetic resources program and other CIAT programs, certifying that it is free of quarantine diseases.



# Functions of GHU-CIAT

- Evaluation and monitoring of pathogens in the regeneration fields.
- Verify the phytosanitary status of the germplasm distributed by GRP and other CIAT programs (beans, forages, and cassava), nationally and internationally.
- Present the certifications required by the Colombian Agricultural Institute (ICA for its acronym in Spanish), Colombian NPPO.
- Carry out research to standardize and implement new diagnosis methodologies that are more efficient and sensitive





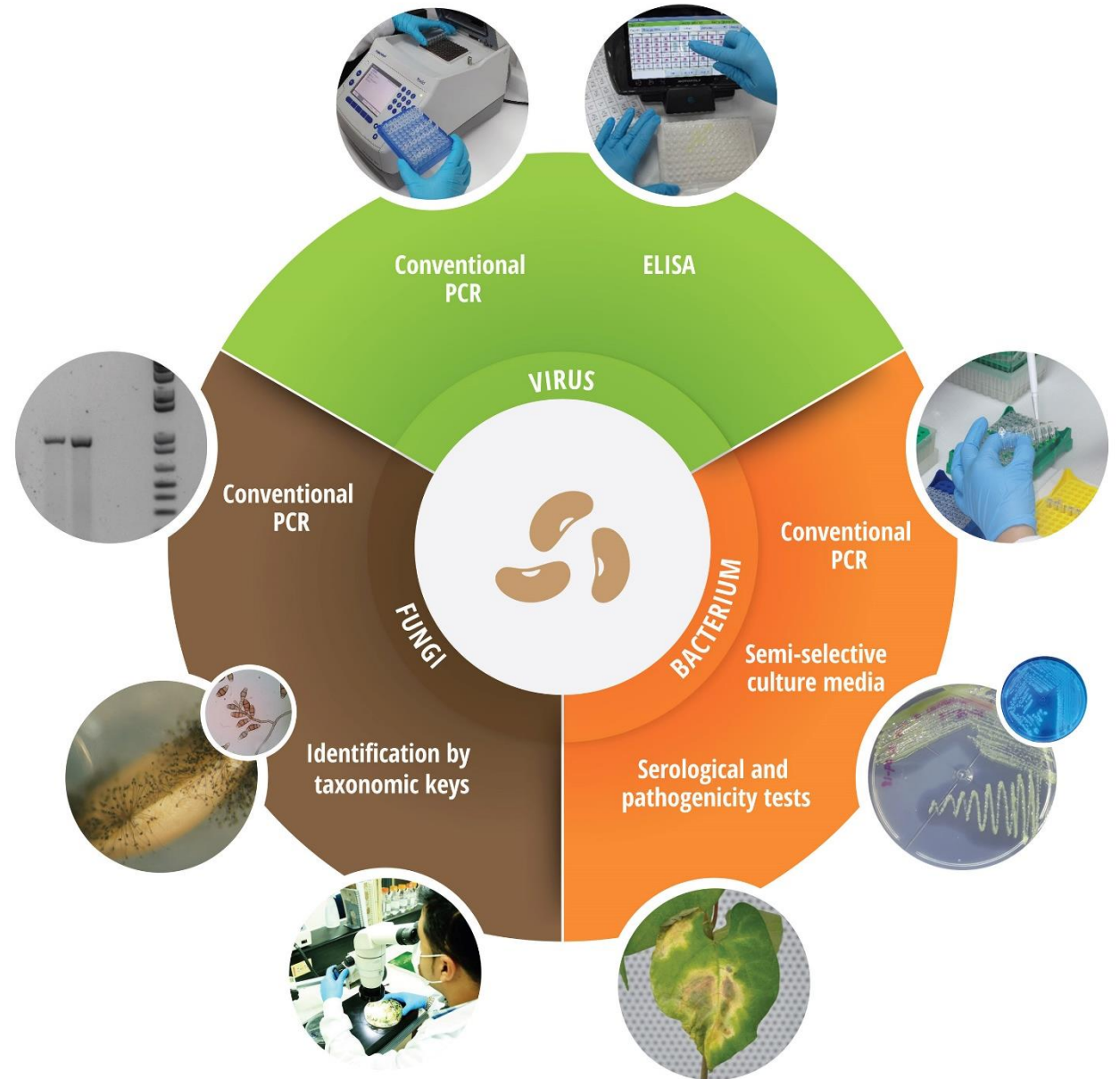
Agronomic  
management and  
phytosanitary  
monitoring in  
regeneration fields



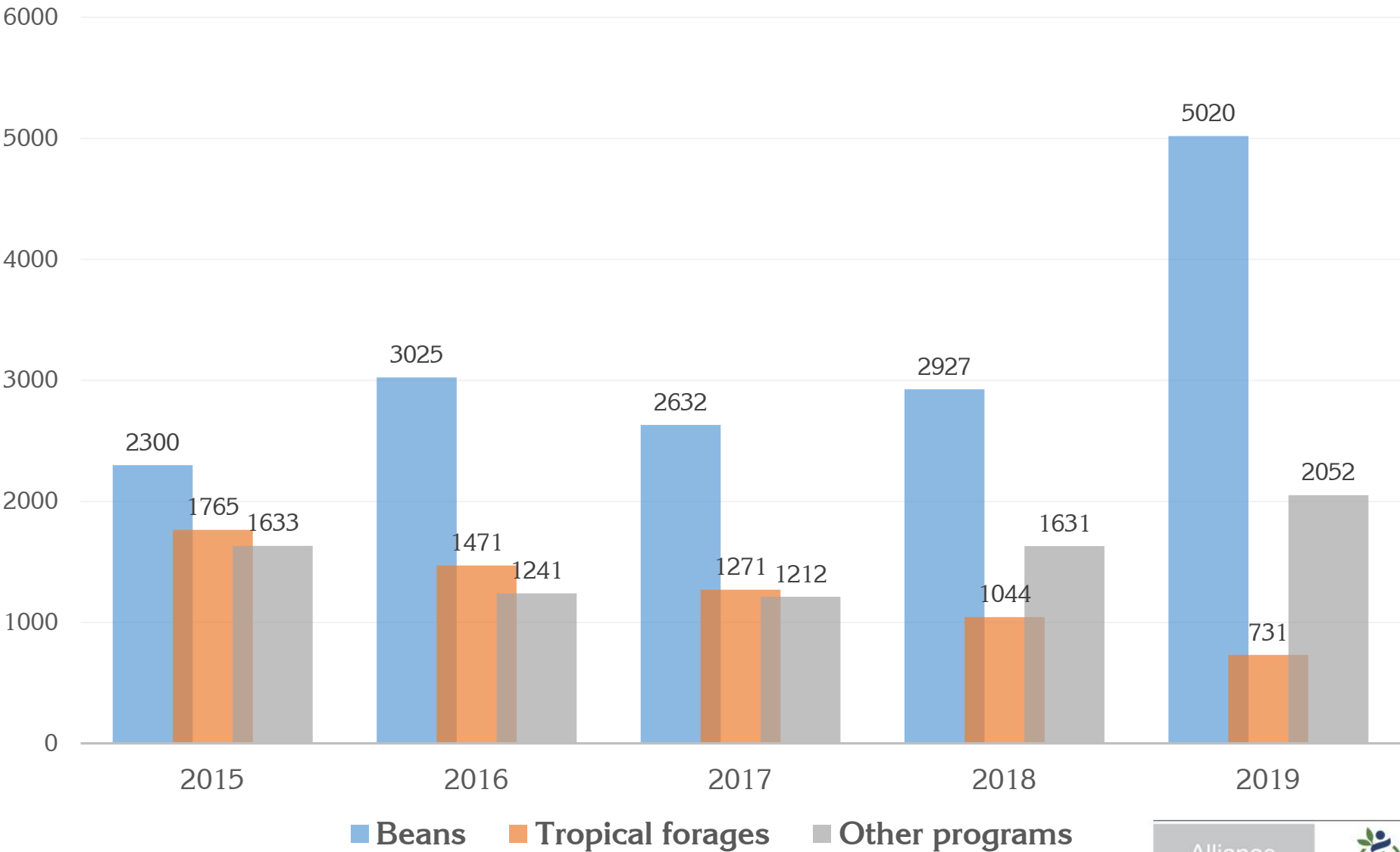
2020, Juan Jose Gonzalez, Javier Gereda.



# SANITARY CERTIFICATION OF BEAN AND FORAGE GERmplasm



# Accessions tested by GHU-CIAT 2015-2019

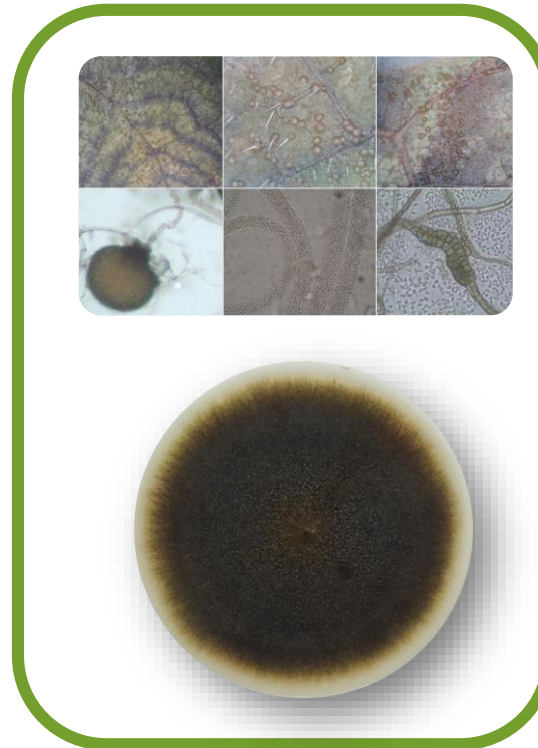


# Diagnostic methodologies for quarantine fungi in beans and forages

## 1. Morphological identification



## 2. Image database and isolation



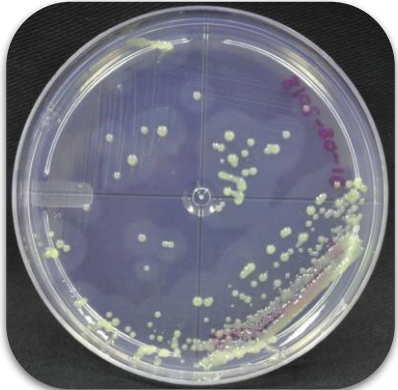
## 3. Confirmatory tests updated to conventional PCR

Region (Locus)	Primers	Primers sequences
ITS *	ITS1	5' - TCCGTAGGTGAACTGCGG - 3'
	ITS4	5' - TCCTCCGCTTATTGATATGC - 3'
Histona 3 **	H3-1a	5' - ACTAAGCAGACCGCCCGCAGG - 3'
	H3-1b	5' - GCGGGCGAGCTGGATGTCCTT - 3'
β-tubulin **	Bt-2a	5' - GGTAACCAAATCGGTGCTGCTTTC - 3'
	Bt-2b	5' - ACCCTCAGTGTAGTGACCCTTGGC - 3'

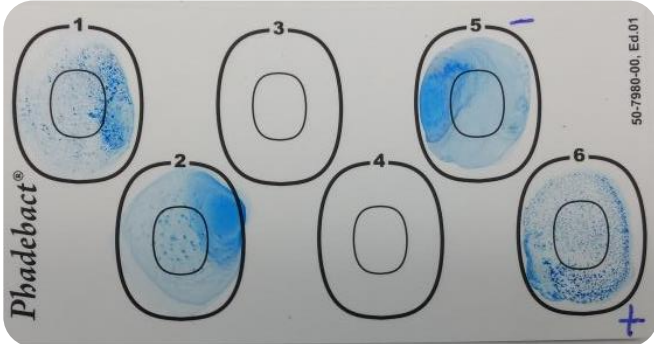


# Diagnostic methodologies for quarantine bacteria in beans and forages

## 1. Isolation in semi-selective media



## 2. Confirmatory tests



Serological test



Pathogenicity test

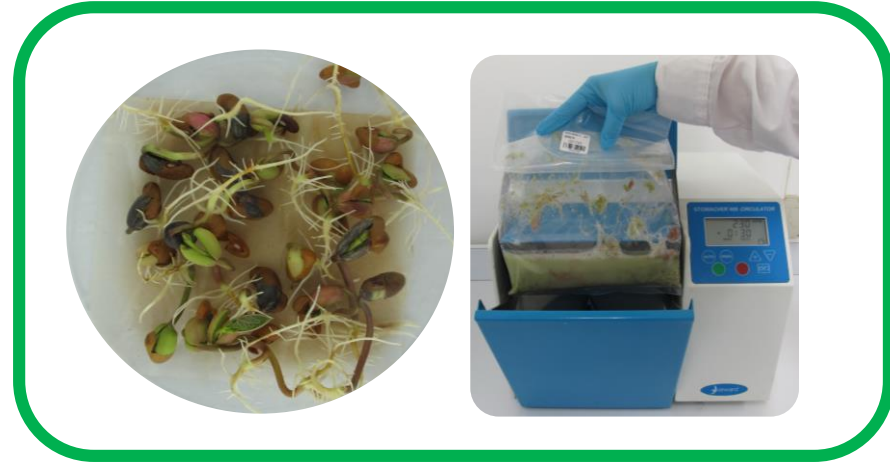
## 3. Confirmatory tests updated to PCR



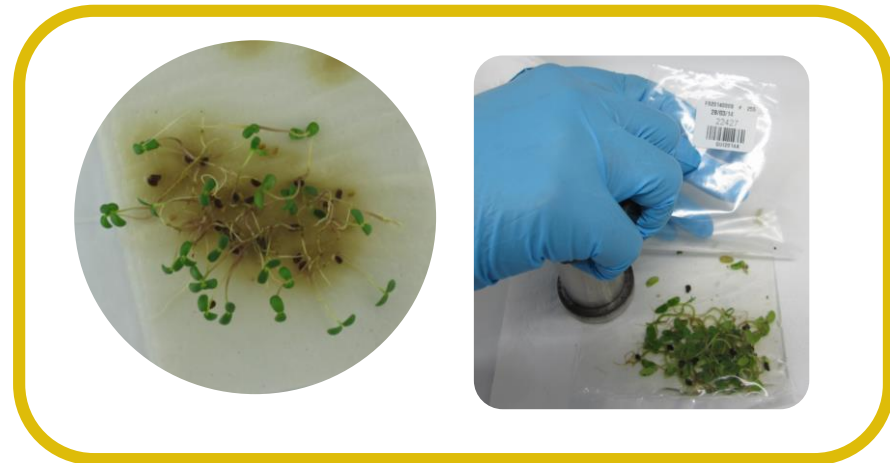
Bacteria	Primers	Primers sequences
<i>Xanthomonas axonopodis</i> pv. <i>phaseoli</i>	X4e	5'-CGCCGGAAGCACGATCCTCGAAG-3'
	X4c	5'-GGCAACACCCGATCCCTAACAGG-3'
<i>Pseudomonas savastanoi</i> pv. <i>phaseoli</i>	HB14f	5'-CAACTCCGACACCAGCGACCGAGC-3'
	HB14r	5'-CCGGTCTGCTCGACATCGTGCCAC-3'
<i>Curtobacterium flaccumfaciens</i> pv. <i>flaccumfaciens</i>	CFFOR2	5'-GTTATGACTGAACTTCACTCC-3'
	CFFREV4	5'-GATGTTCCCGGTGTTTCAG-3'

# Methodologies for the diagnosis of viruses in beans and forages

## 1. Grind plant tissue

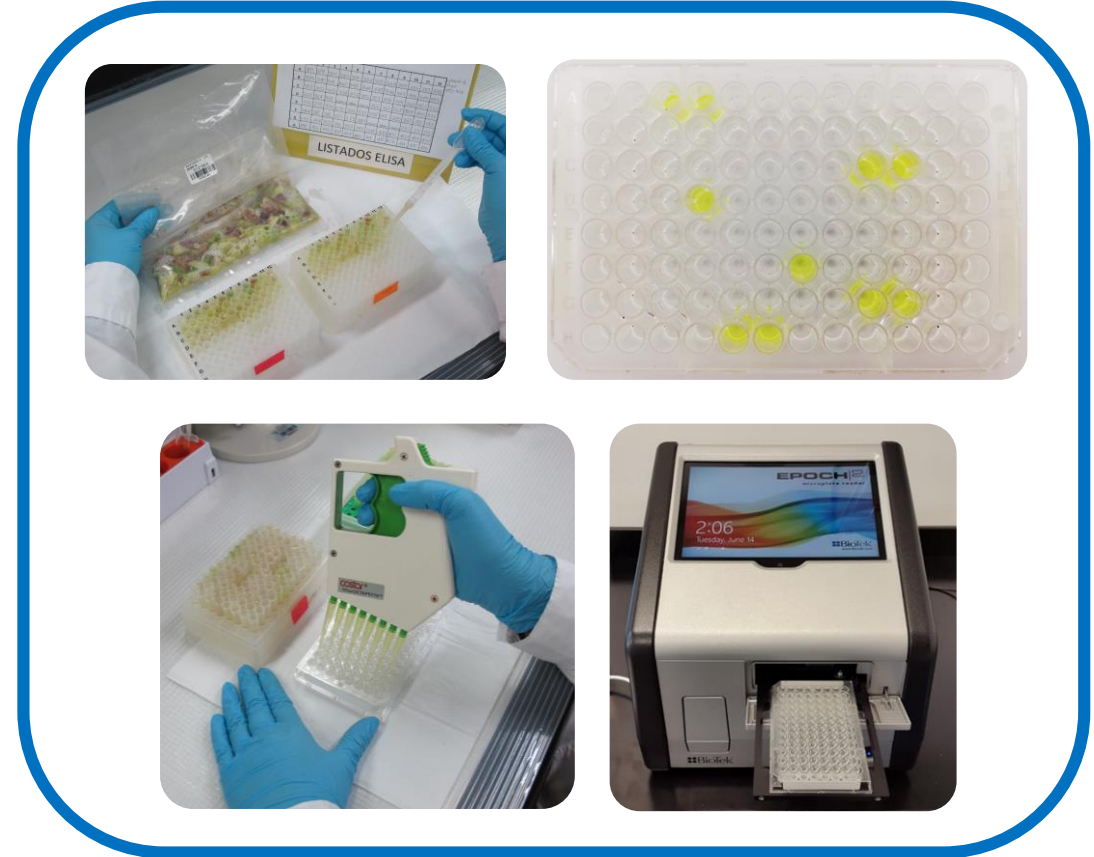


Beans



Tropical forages

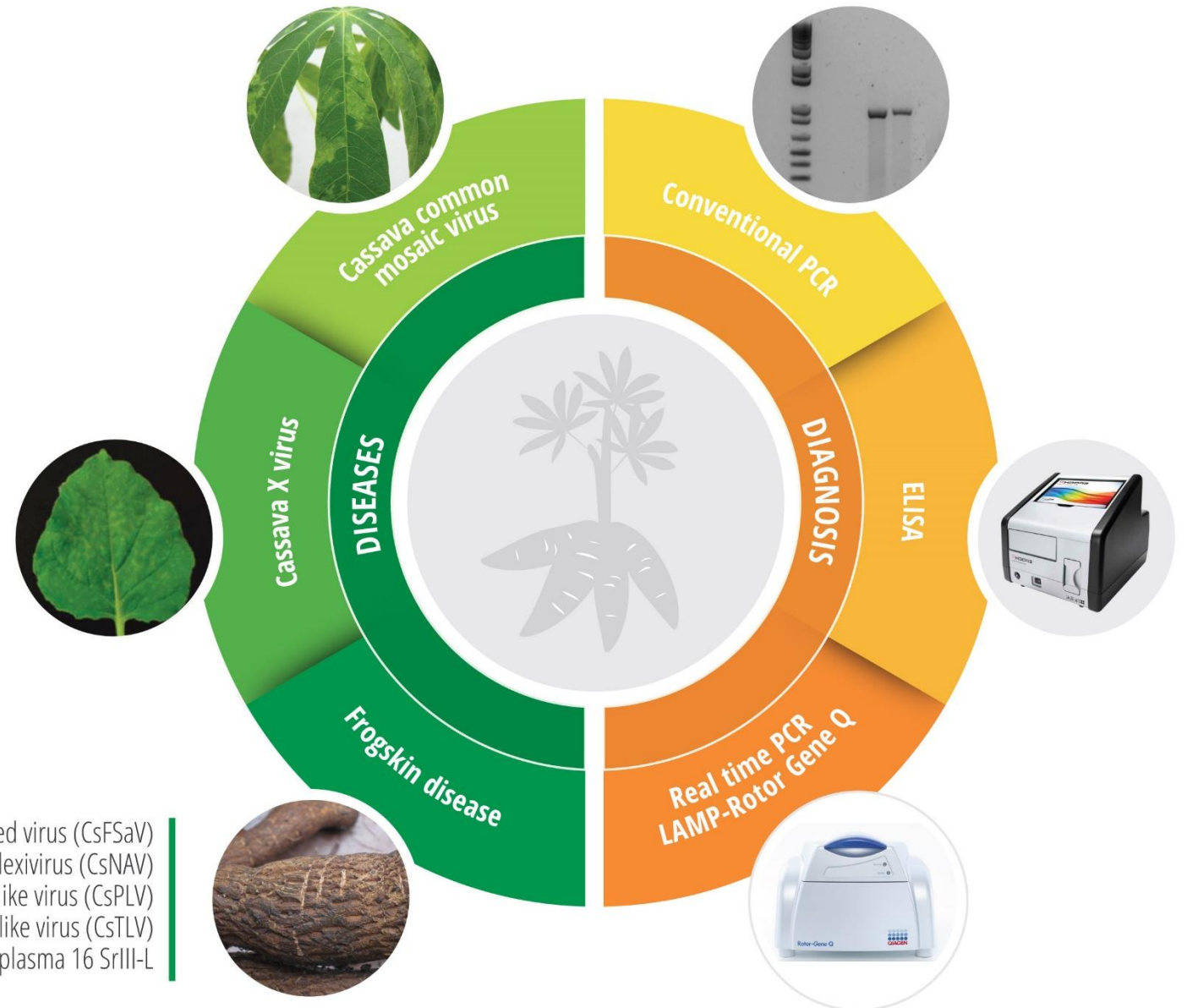
## 2. ELISA test



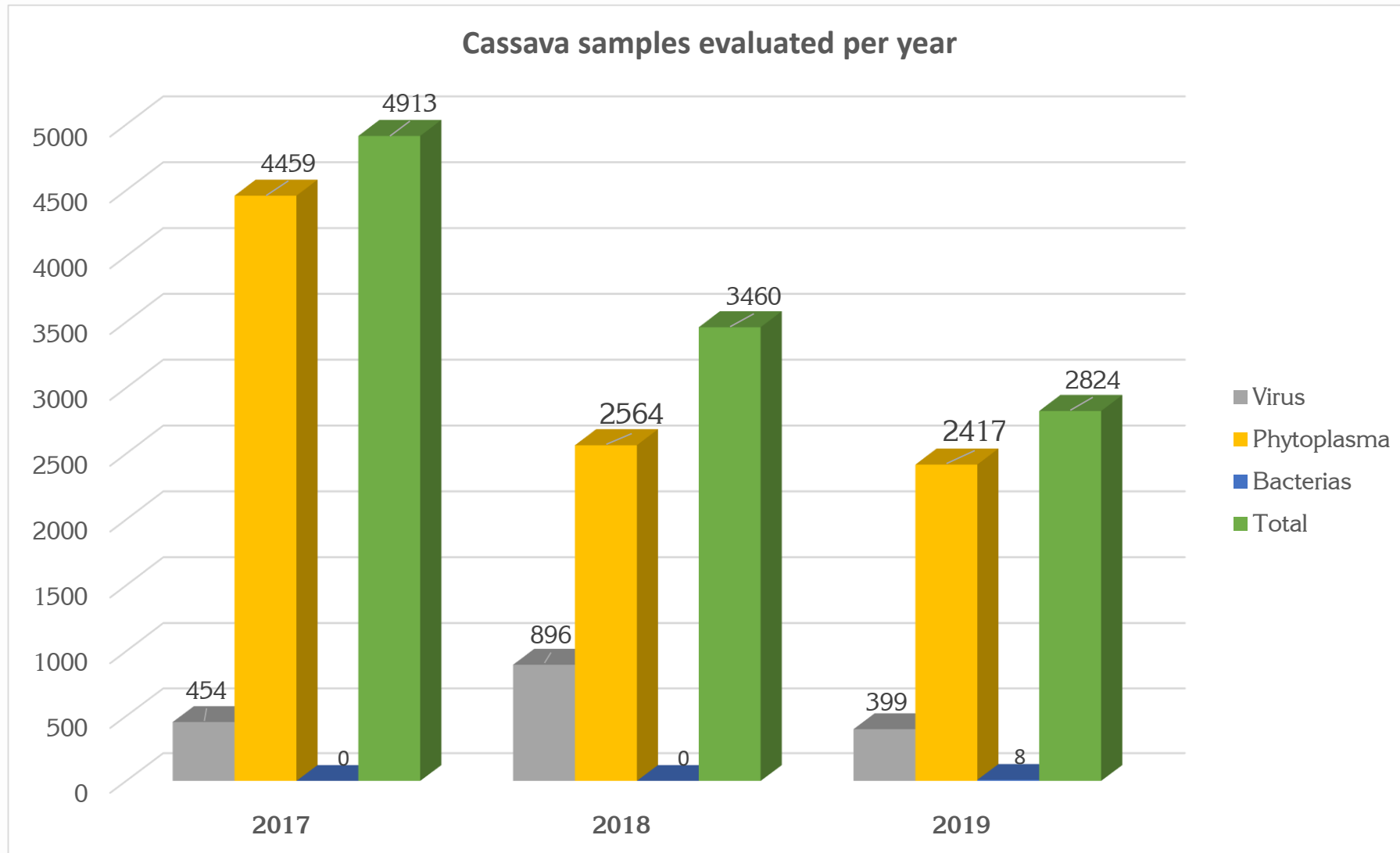


# SANITARY CERTIFICATION OF CASSAVA GERMPLASM

- Cassava frogskin associated virus (CsFSaV)
- Cassava new alphaflexivirus (CsNAV)
- Cassava polero-like virus (CsPLV)
- Cassava Torrado-like virus (CsTLV)
- Fitoplasma 16 SrIII-L



# Cassava samples evaluated per year



# Sanitary certification of viruses in Cassava

1. Reception of plant material



*In vitro* plants with leaf tissue

2. RNA nucleic acid extraction

Protocol:  
CTAB Modified  
(Lopez et al., 2006)



3. Nucleic acid concentration quantitation (RNA)

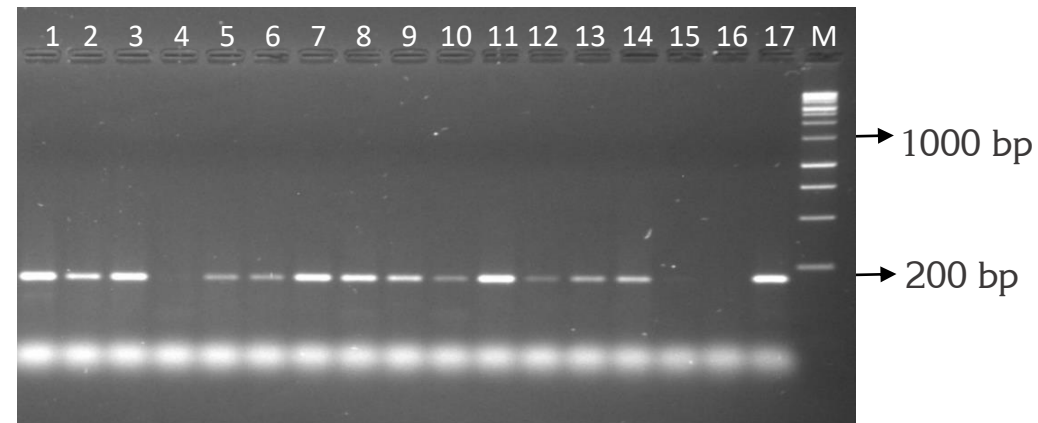


cDNA verification

4. cDNA synthesis with Random primer



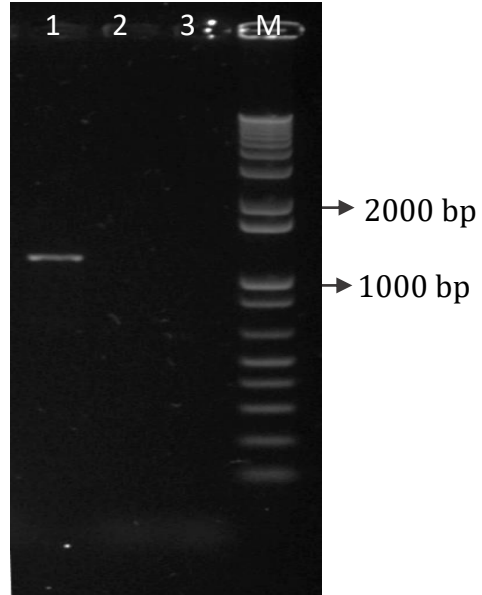
5. PCR Internal Control (Designed by USDA)



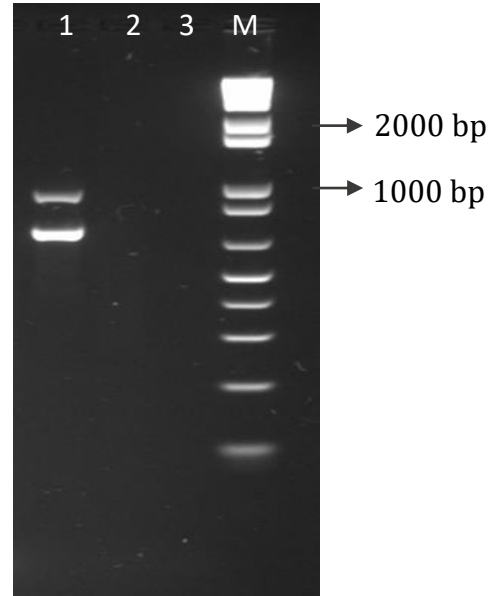
# Sanitary certification of viruses in Cassava



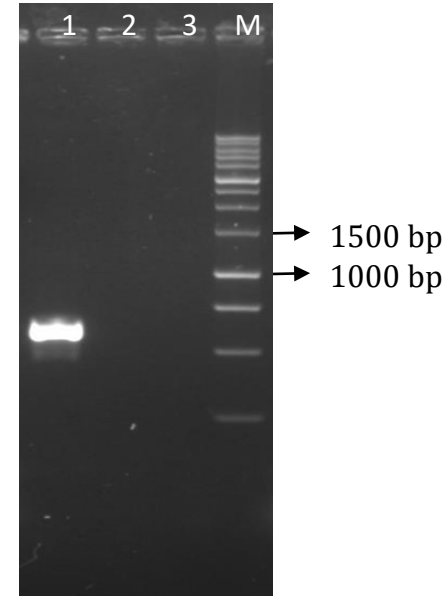
PCR



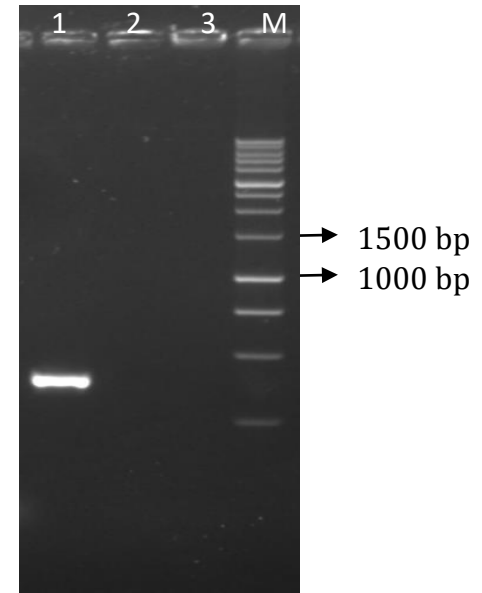
**\*CsNAV**  
1600 bp



**\*CsTLV**  
RNA 1: 850 bp  
RNA 2: 720 bp



**\*\*CsCMV**  
647 bp



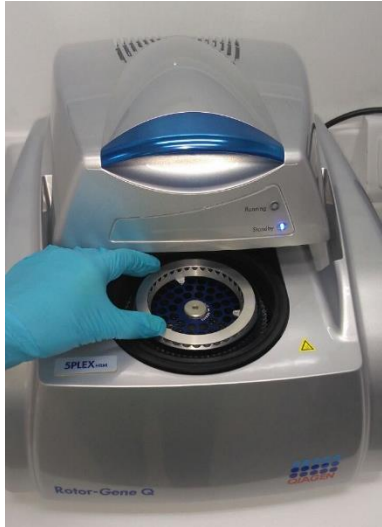
**\*\*CsXV**  
408 bp

## 6. Diagnosis of quarantine viruses using conventional PCR

\* Carvajal et al., 2014

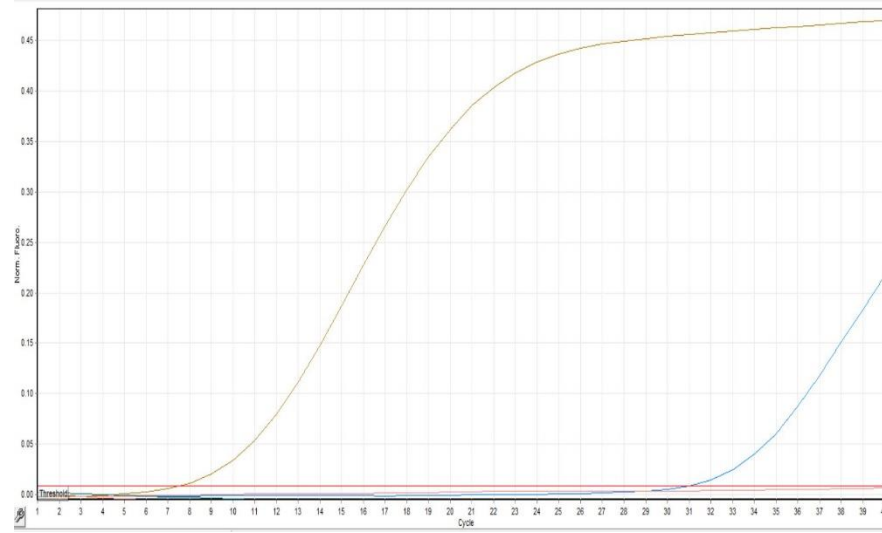
\*\* Lozano et al., 2017

# Sanitary certification of viruses in Cassava



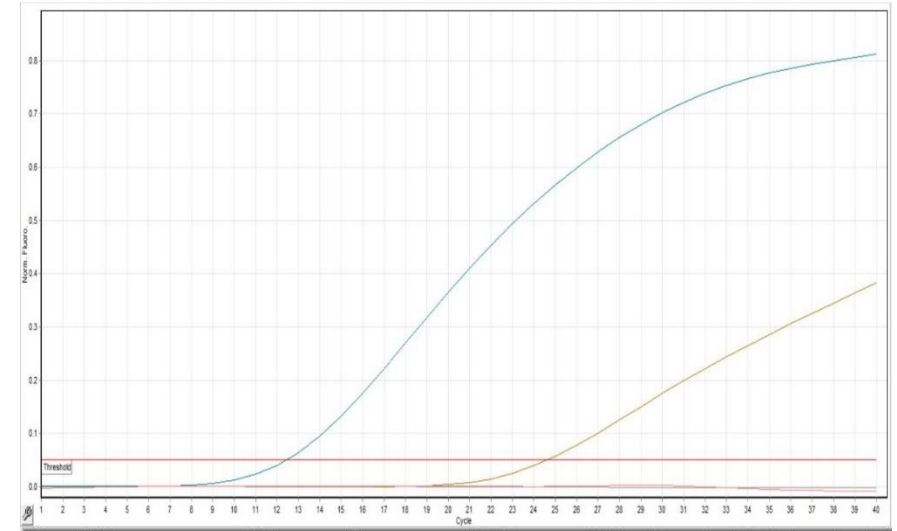
qPCR

\*CsFSaV



No.	C	Name	Type	Ct	Ct Comment	Given Conc (cop)	Calc Conc (copie)
38	87		Unknown	31.07			2233917153E-09
53		Control Positivo	Positive C	7.60			5494881275E-03
71		Control Negativo	Negative				
72		Blanco	NTC				

\*\*CsPLV



No.	C	Name	Type	Ct	Ct Comment	Given Conc (cop)	Calc Conc (copie)
53	193		Unknown	24.72			9639724329E-04
62		Blanco	NTC				
71		Control Positivo	Positive C	12.49			17867557857122
72		Control Negativo	Negative				

## 7. Diagnosis of quarantine viruses using qPCR

\*Carlvert et al., 2008; Cuervo, 2006; Pardo, 2013

\*\*Carvajal et al., 2014

# Sanitary certification of Phytoplasma 16SrIII-L in Cassava



1. Reception of plant material

*In vitro* plants with leaves, roots and stem



2. DNA nucleic acid extraction

Protocol:  
CTAB modified  
(Murray & Thompson, 1980)



3. Nucleic acid concentration quantitation (DNA)

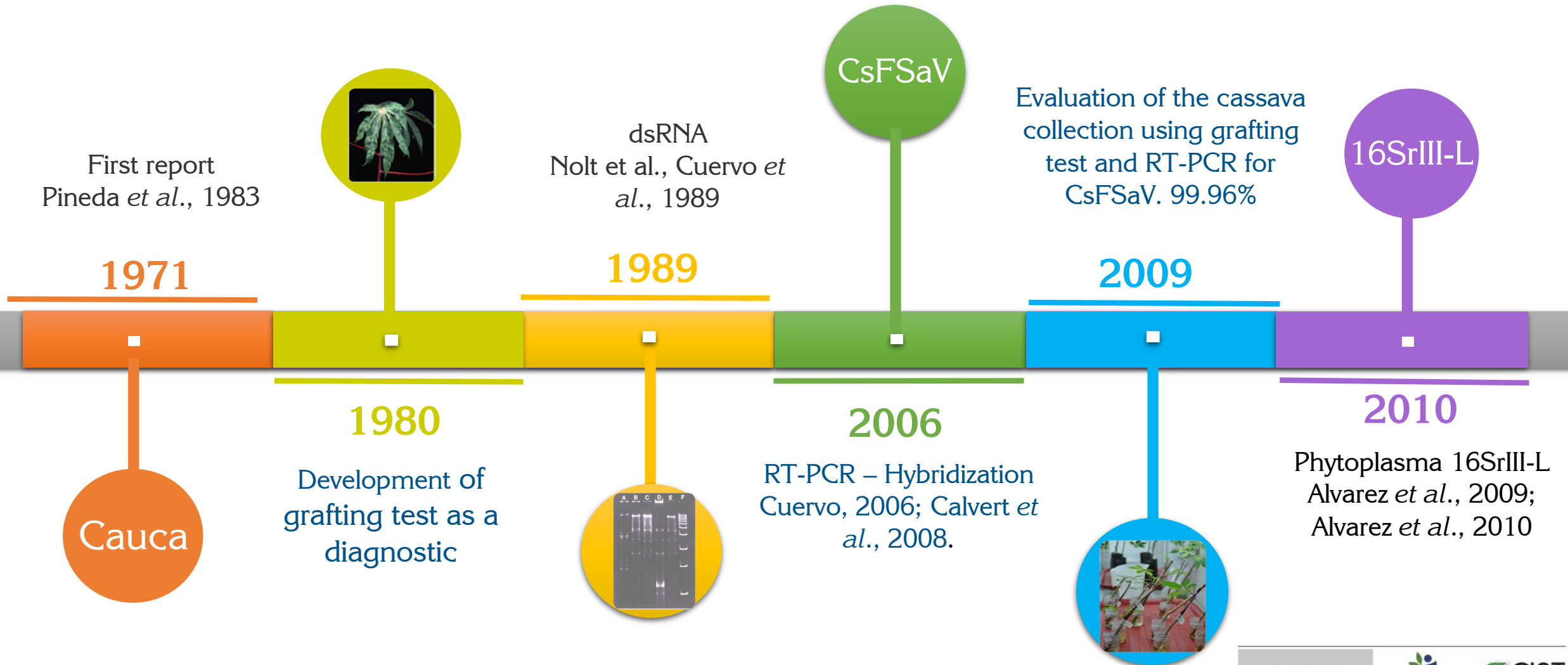


4. Phytoplasma 16Sr III-L Diagnosis using LAMP – Rotor Gene Q

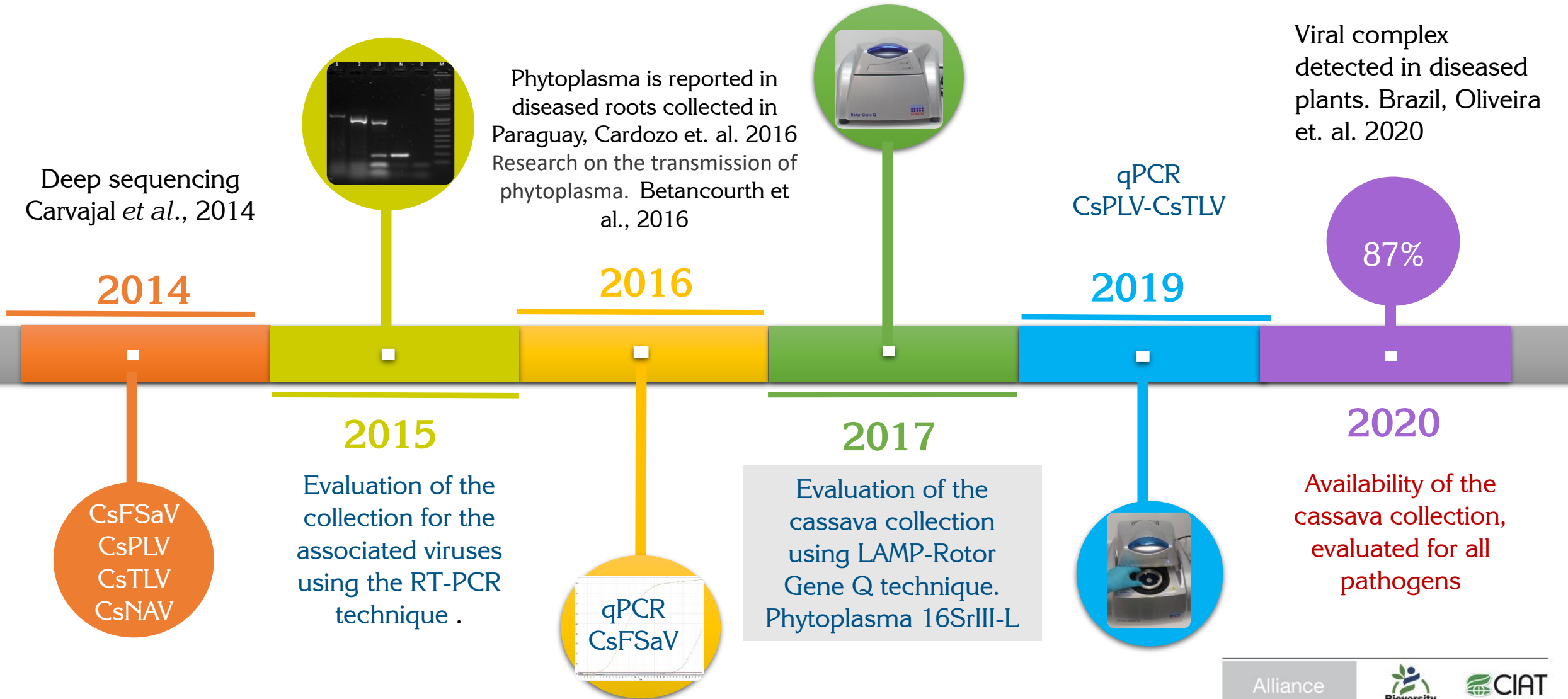
Alvarez *et al.*, 2009; Alvarez *et al. et al.*, 2014; Keremane 2015



# Diagnostic methodologies of pathogens associated with cassava frogskin disease



# Diagnostic methodologies of pathogens associated with Frogskin disease



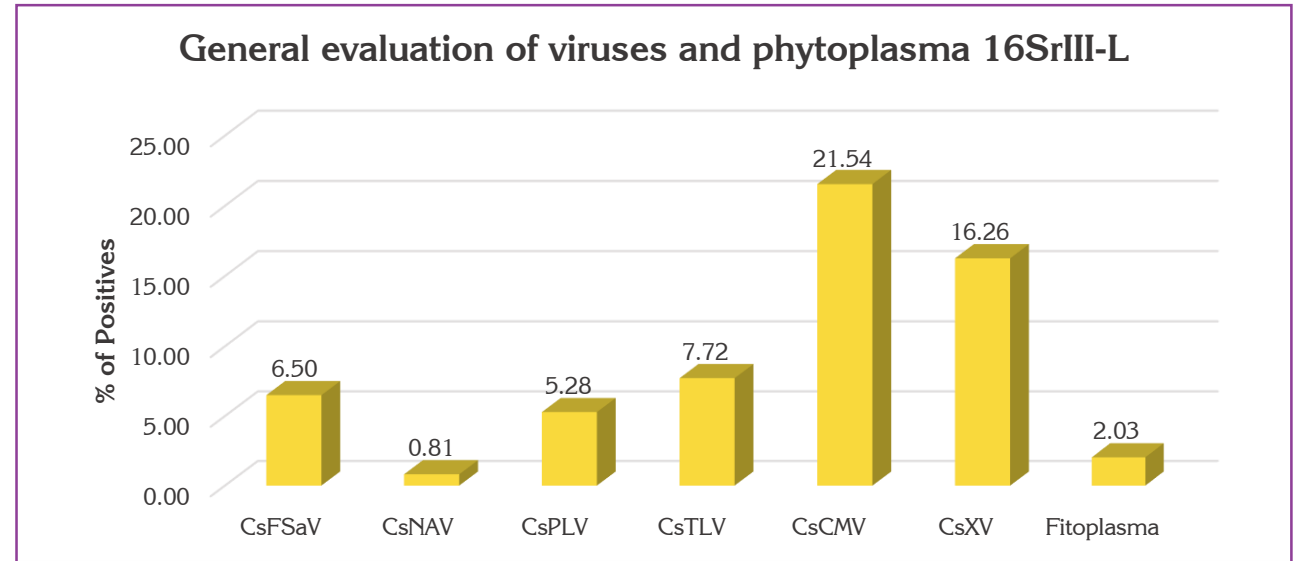
# Progress of the sanitary evaluation of Cassava collection

The use of different diagnostic methodologies has allowed the evaluation of the collection for pathogens of quarantine interest (Virus and phytoplasma 16SrIII-L).

Achieved to date (Oct. 2020): **87 %** availability

Accessions: **6.155**

# Sanitary evaluation of the “cassava bonsai collection”

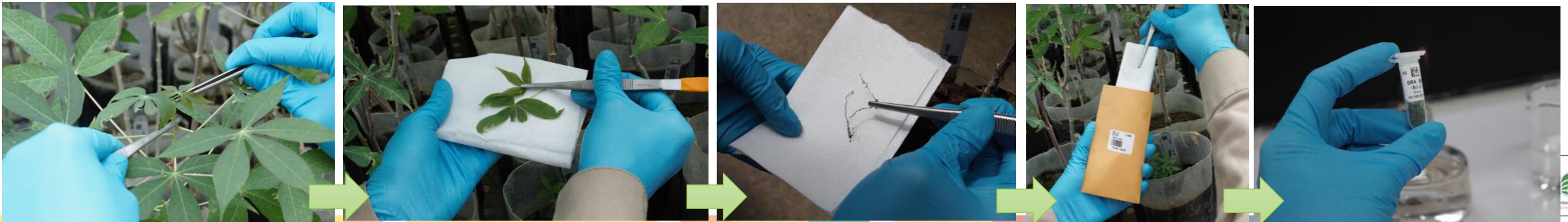


D. Nino, L.J. González, 2020

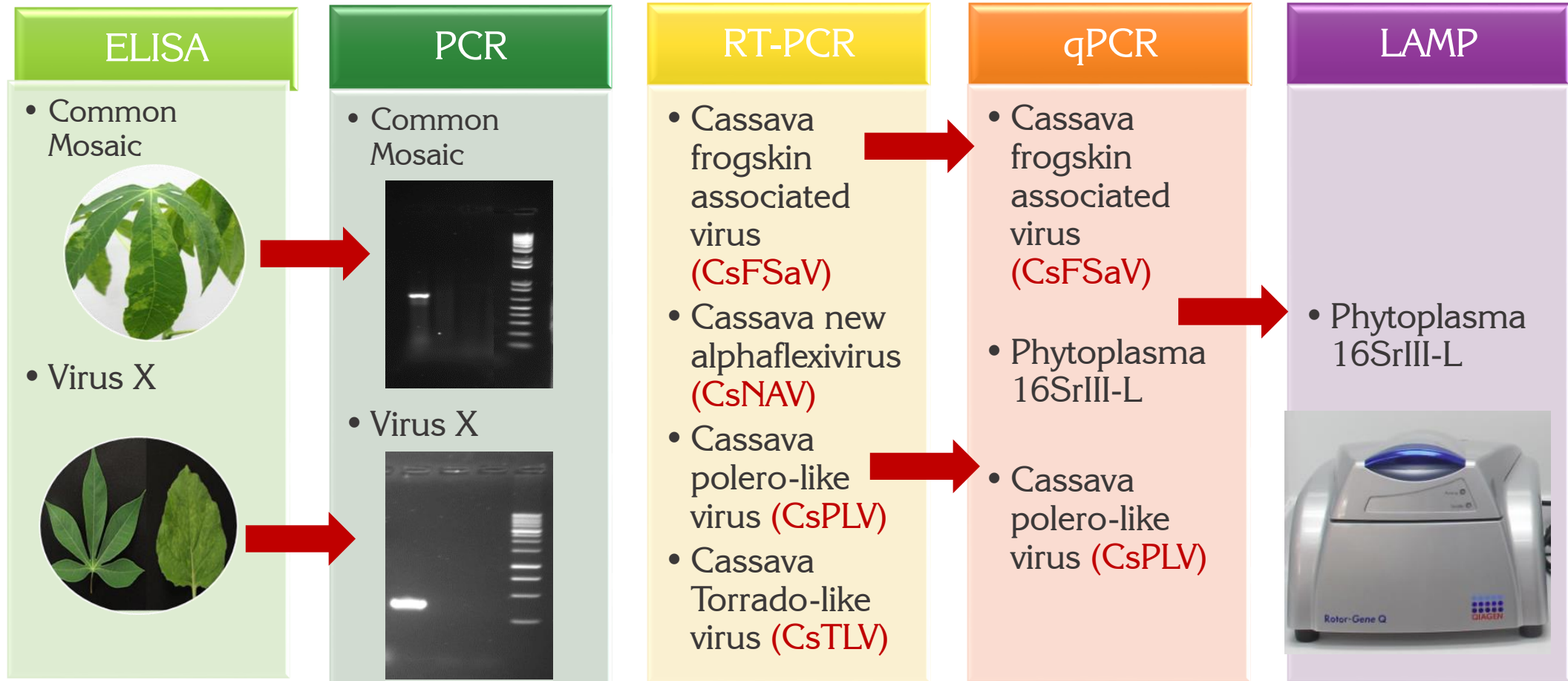
Accessions collected: 496

Accessions evaluated: 246 / Accessions in process: 250

## Sampling of plant material for the sanitary evaluation of the greenhouse



# Implementation of new diagnostic methodologies in Cassava



**Future plans and implementation of new diagnostic methodologies for the sanitary evaluation of cassava**

**Frogskin disease:**  
CsFSaV / CsNAV /  
CsPLV / CsTLV

Implementation of  
Multiplex Real-  
Time PCR (qPCR)



**Cassava common  
mosaic and virus X**  
CsCMV y CsXV

Probe and  
Primer Design  
for Multiplex  
qPCR



**Deep sequencing**

Diagnosis and  
confirmation of  
positive samples,  
detection of new  
pathogens

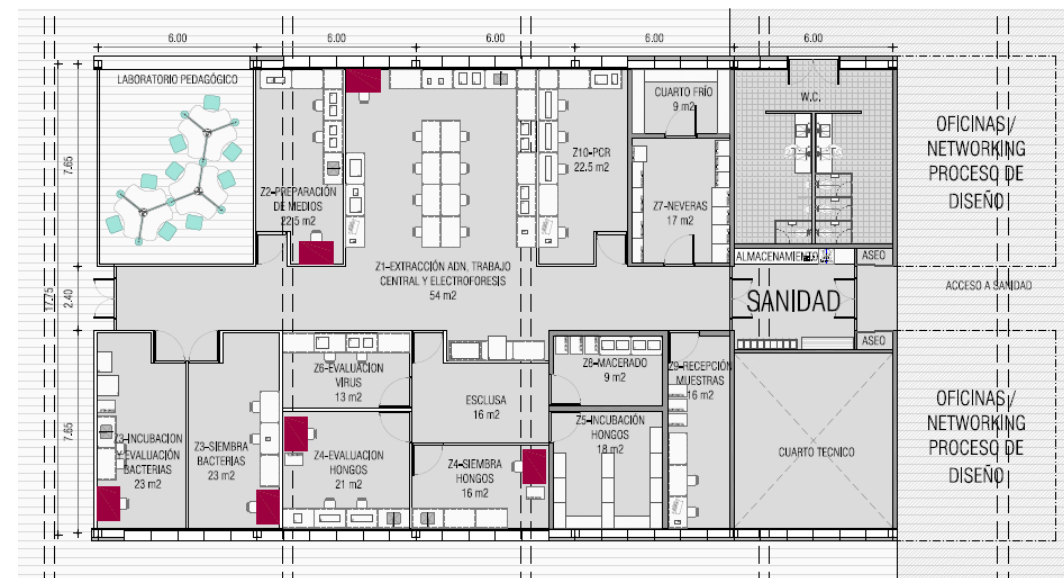


# “FUTURE SEEDS” GeneBank new building



GHU-CIAT Laboratory  
Actual area: 130.68 m<sup>2</sup>  
New area: **415.33 m<sup>2</sup>**

Future Seeds includes the new GHU facilities with improved areas and increase diagnostic capacity.





# Thanks!