



Development of an *in situ* hybridization method to detect and localize cassava brown streak virus in tobacco and cassava hosts based on RNAscope® technology



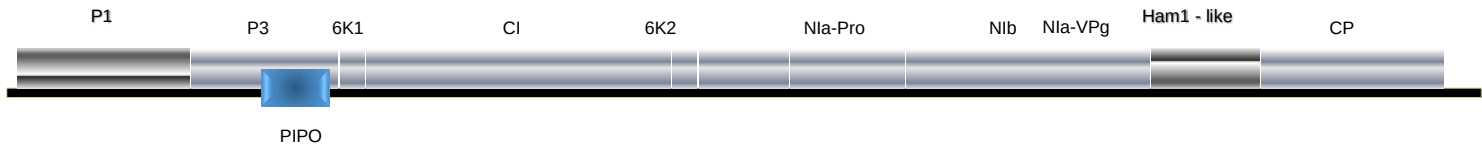
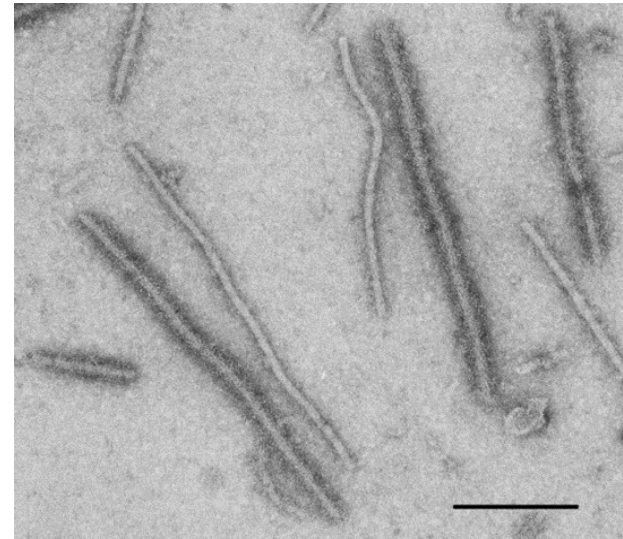
Esperance Munganyinka, Paolo Margaria, Samar Sheat, Elijah Ateka, Fred Tairo, Joseph Ndunguru, Stephan Winter

Outline

- ❑ Cassava brown streak viruses (CBSVs)
- ❑ Diagnosis of CBSVs
- ❑ *In situ* hybridization (ISH) RNAscope technology
 - Experiments
 - Results
 - Conclusion

Cassava brown streak viruses

- **CBSV** & **UCBSV** (Potyviridae, *Ipomovirus*)
 - Positive single-stranded RNA (+ssRNA) viruses
 - Distinct species
 - U/ CBSV nt similarity ~70%
 - Genome sizes: 8900-9070 nt
 - Transmitted by *Bemisia tabaci* & by infected cuttings



CBSVs are the most devastating pathogens of cassava



Diagnosis of CBSVs

Molecular and serological methods are available for detection, discrimination and quantification of CBSVs

ELISA, RT-PCR, RT-qPCR

Challenges

Uneven distribution of the virus in the plant:

- Quantification of virus in cassava leaves showed high fluctuation of concentrations between leaf stages as well as between cassava genotypes
- Cassava genotypes may have low virus load in leaves but still have considerable virus concentrations in tubers (Ogwok et al., 2015)

Research question

Are there any differences in affinity/tropism of CBSV to invade particular tissues or a limited suitability of some host tissues for virus replication?

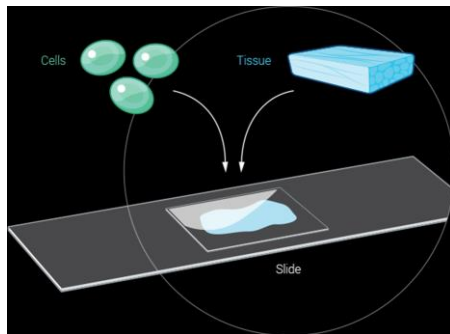
Objectives

To develop a method that allows the sensitive detection and localization of CBSV in cassava organs and tissues

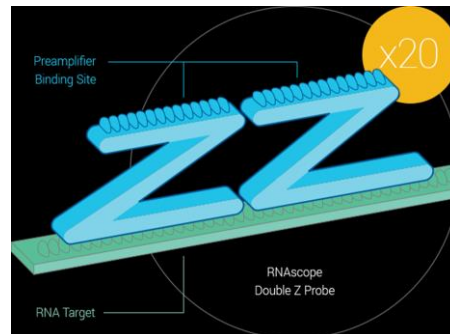
In situ hybridization RNAscope technology

- a novel ISH assay introduced in 2011 for detection of target RNA within cells
- represents a major advance with its proprietary probe design to amplify target-specific signals and background suppression

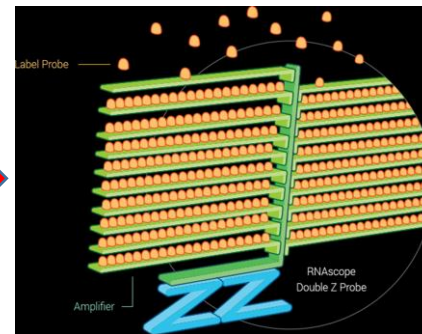
RNAscope *in situ* hybridization assay workflow



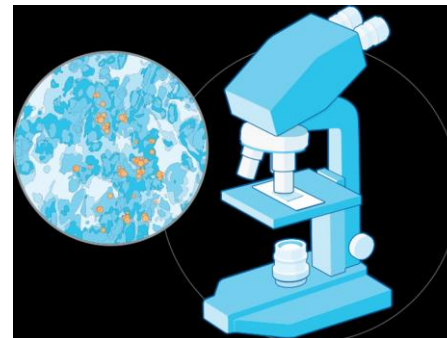
Permeabilize



Hybridize



Amplify



Visualize



Quantify

Experiments: Plant material and virus infections with **CBSV Mo 83**

Nicotiana rustica:
14 days post
inoculation



Cassava TME7:
5 months after
grafting



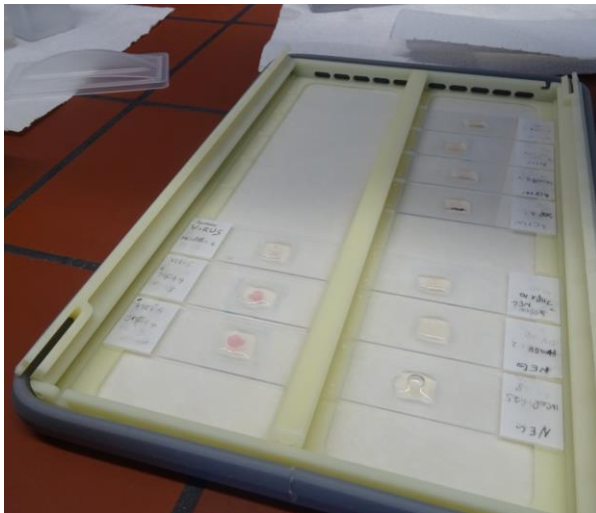
Sample preparation



Fixation (Formalin)



Embedding (Paraffin)

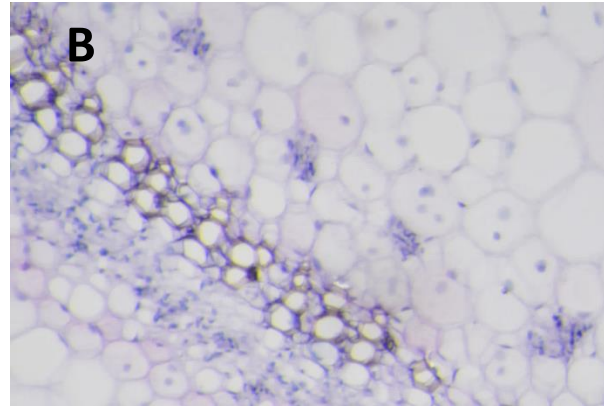
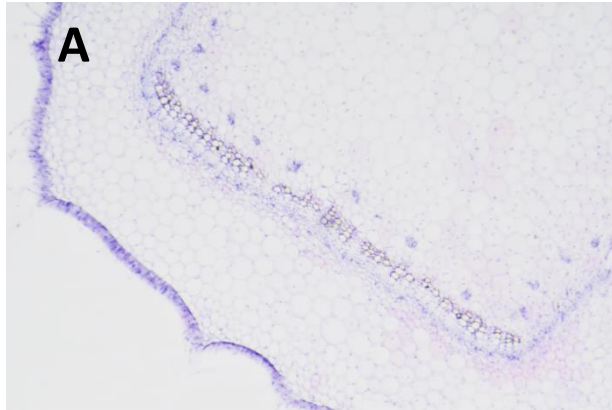


Hybridization

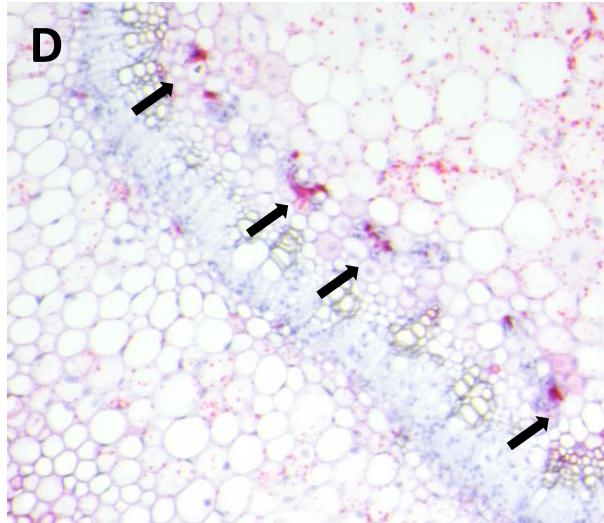
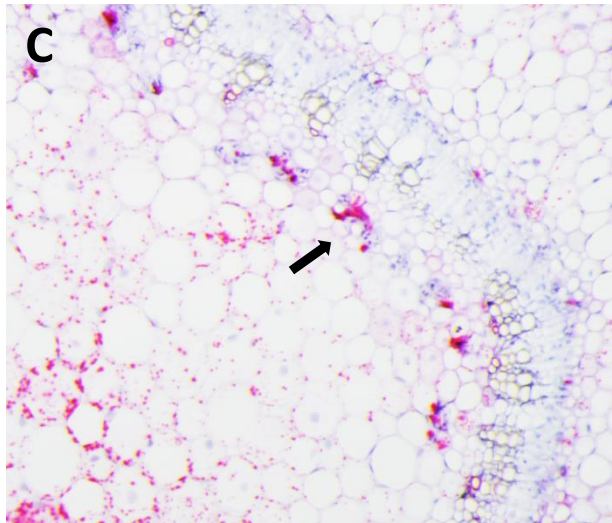


Sectioning (10 μ m)

Results: Localization of CBSV-Mo83 RNA in cross sections of *Nicotiana rustica* stem tissues



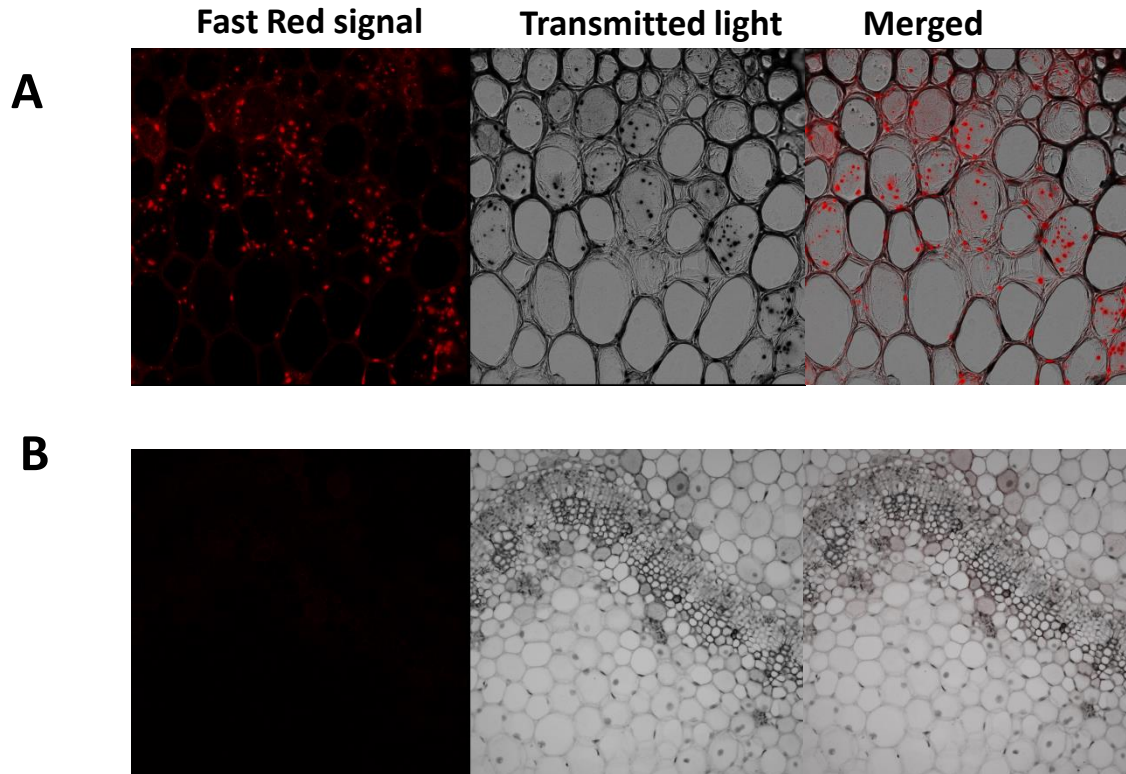
(A,B) Tissue from healthy control plants.



(C,D) Tissue from plants infected with CBSV-Mo83.

Abundant presence of viral RNA in cells visualized as single red dots or dots merged into signal clusters (arrows).

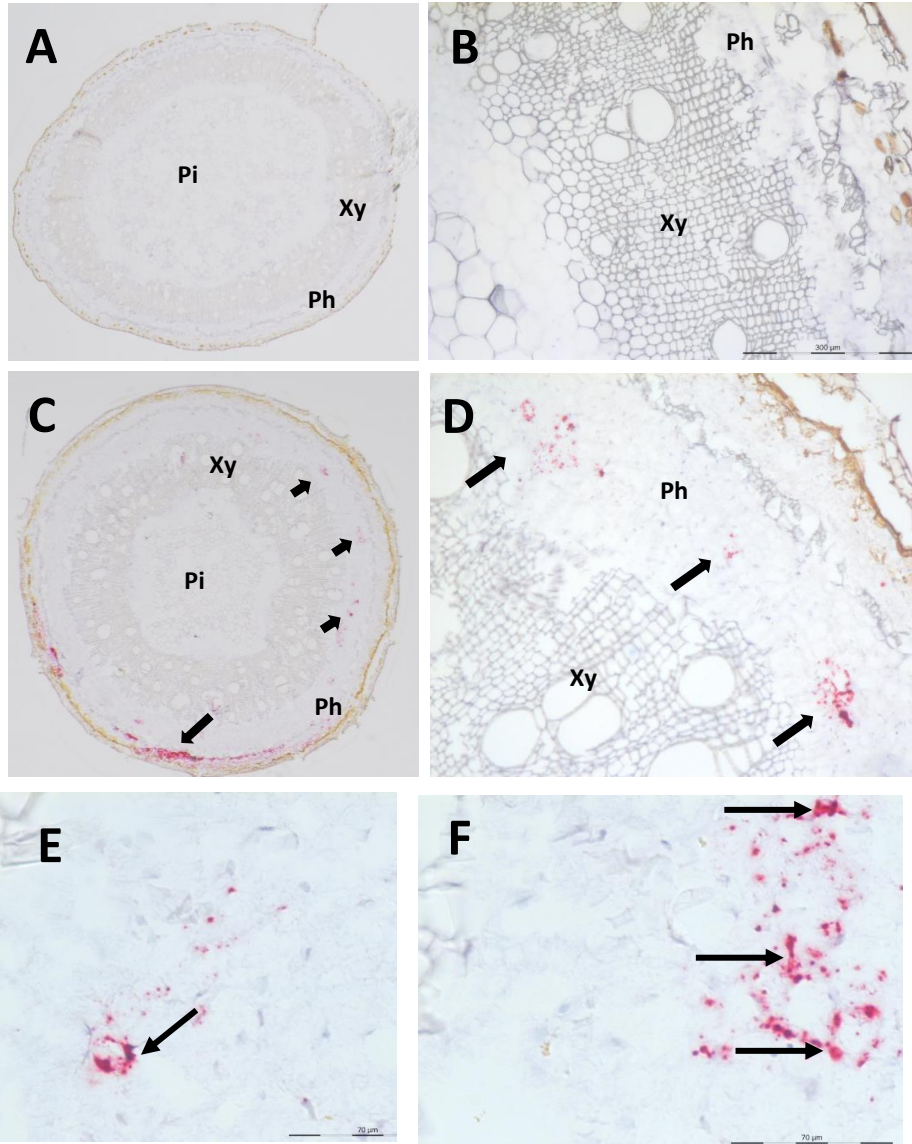
Illustration of CBSV-Mo83 RNA accumulation in infected *Nicotiana rustica* cells using confocal laser scanning microscopy



(A) Fast Red signal (left) laid over image from transmission results into merged picture (right), to demonstrate virus in an infected cell.

(B) Non-infected control.

Localization of CBSV-Mo83 RNA in cross sections of cassava stem tissues



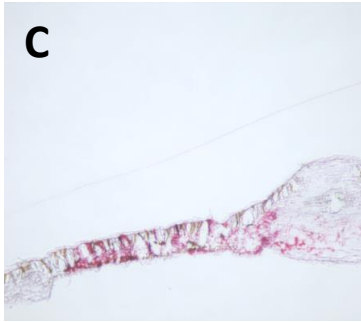
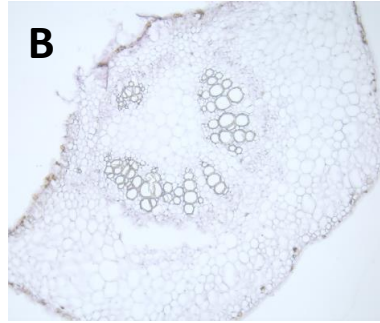
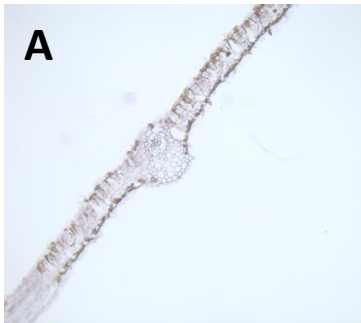
(A,B) Sections from a control healthy plant.

(C-F) Stem sections from CBSV-Mo83 infected cassava.

Red dots indicate hybridization of the probes to viral RNA. Accumulation of dots mostly in phloematic cells (arrows in C and D).

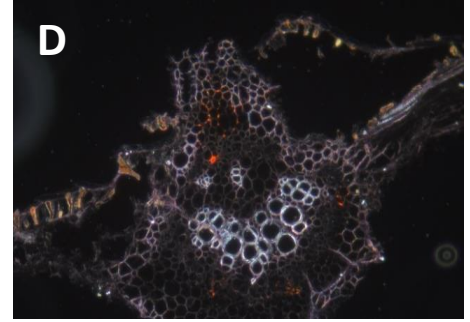
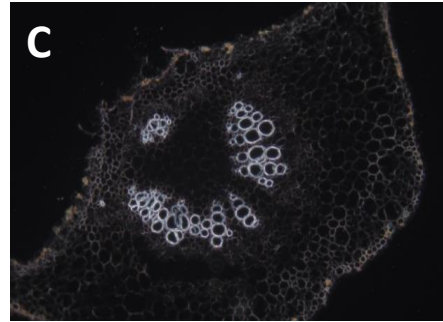
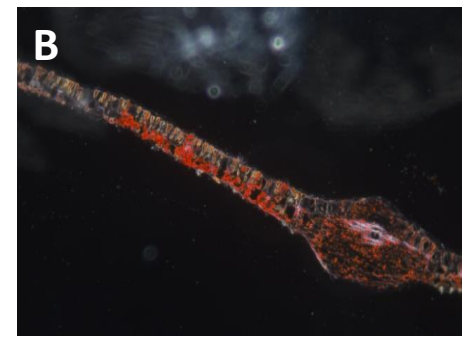
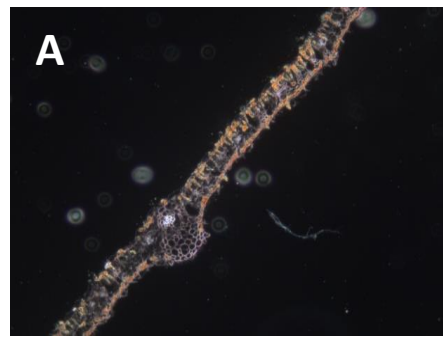
Signal clusters were occasionally detected (arrows in E and F).

Localization of CBSV-Mo83 RNA in cross sections of cassava leaves



Lamina and midrib tissues of healthy (A,B) and infected leaves (C,D).

Abundant presence of viral RNA in cells visualized as red dots, with absence of hybridization signal in healthy controls.



Differential Phase Contrast imaging:

(A,C) healthy control sections

(B,D) CBSV-Mo83 infected section.

Hybridization signal appears as shining orange color.

Conclusions

- ❑ We provide an ISH RNAscope method for detection and localization of CBSV in tissue sections from *N. rustica* and cassava
 - *The entire process can be completed in 4 days for rustica and 5 days for cassava*
 - *Difficulty in sectioning cassava tissues compared to N. rustica*
 - *Viral RNA was highly abundant in phloematic and non-phloematic tissues of N. rustica*
 - *The signal appeared more localized around phloematic tissues in cassava*
- ❑ The ISH RNAscope assay provides a highly sensitive method to localize even minute amounts of virus RNA in infected plant tissues
- ❑ We will use the ISH RNAscope method for investigating other virus–host combinations and co-localization

Acknowledgements

Joseph Ndunguru

Stephan Winter

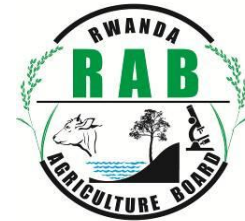
Elijah Ateka

Paolo Margaria

Fred Tairo

Samar Sheat

DSMZ-Plant Virus Department Research group



Thank You!

