# Maize lethal necrosis in East Africa : tracking an emerging disease using NGS

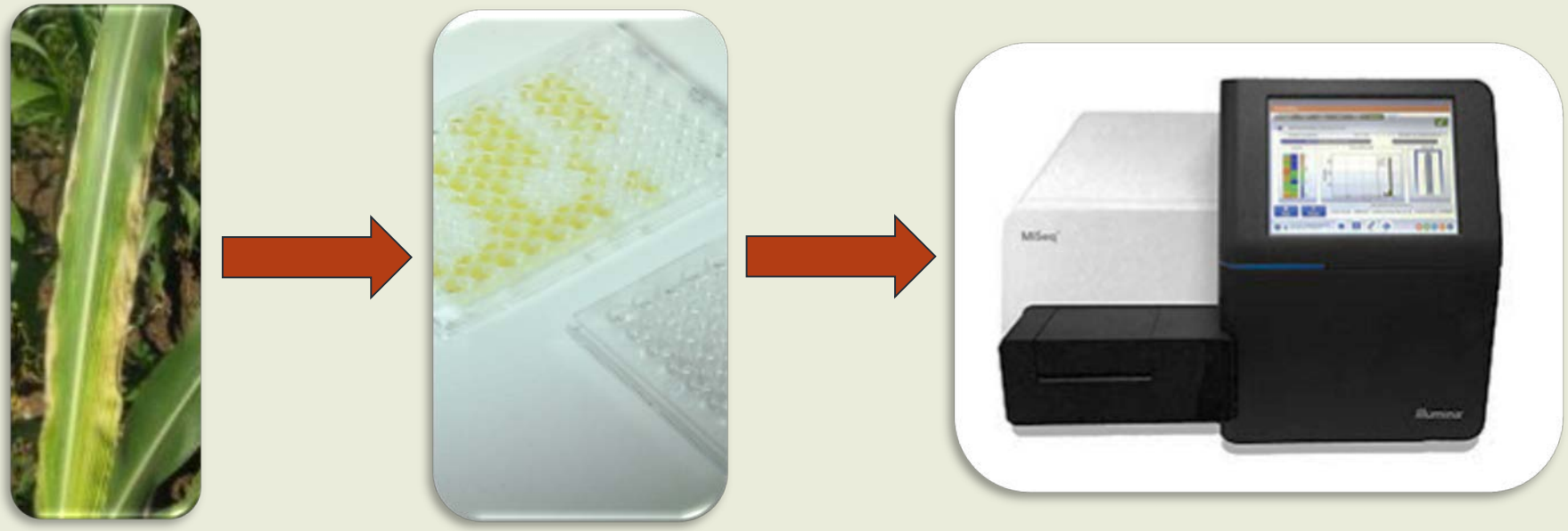
Neil Boonham, Ian Adams, Adrian Fox and Julian Smith

Detection and Surveillance Technologies team
Fera Science Ltd

# Maize diseases in East Africa

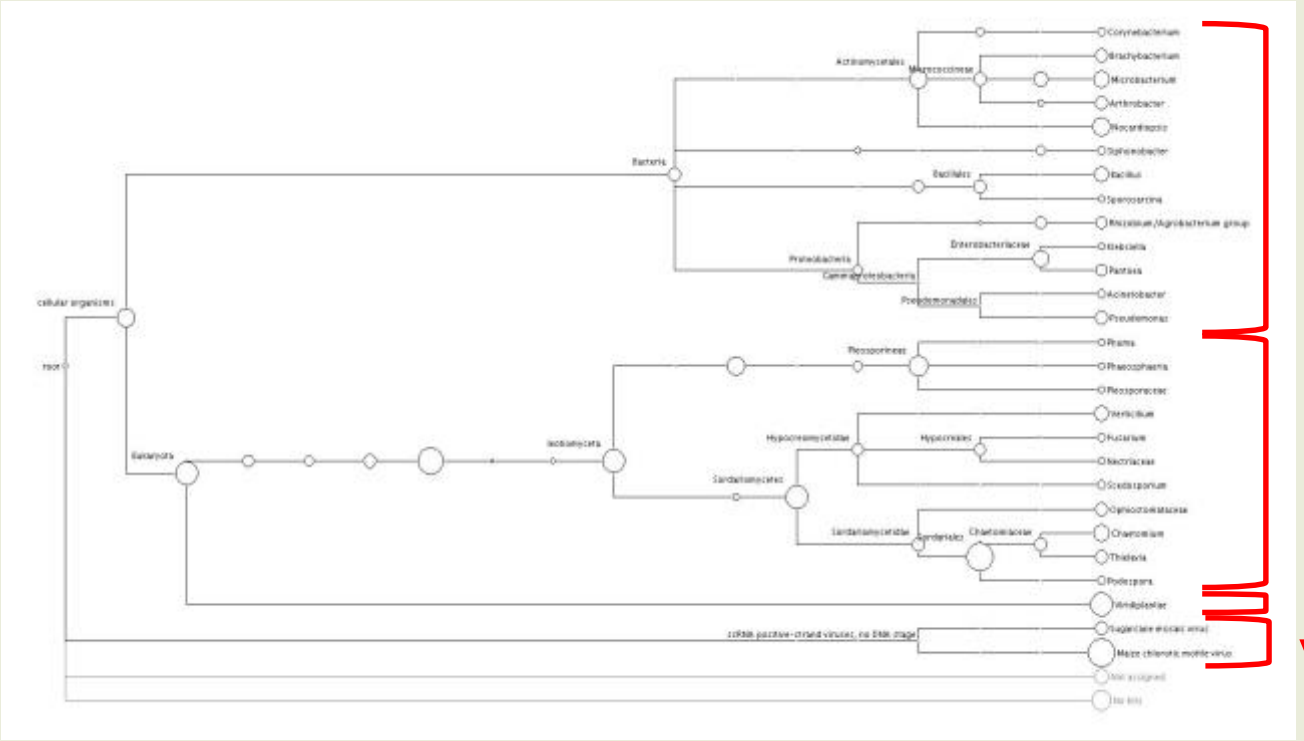
- New disease noted in 2011
- Samples tested in various labs in Africa/US
- No conclusive agent detected

# Samples tested using NGS



Adams IP1, Glover RH, Monger WA, Mumford R, Jackeviciene E, Navalinskiene M, Samuitiene M, Boonham N. (2009) Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. Mol Plant Pathol. 2009 Jul;10(4):537-45. doi: 10.1111/j.1364-3703.2009.00545.x.

# Identified two viruses



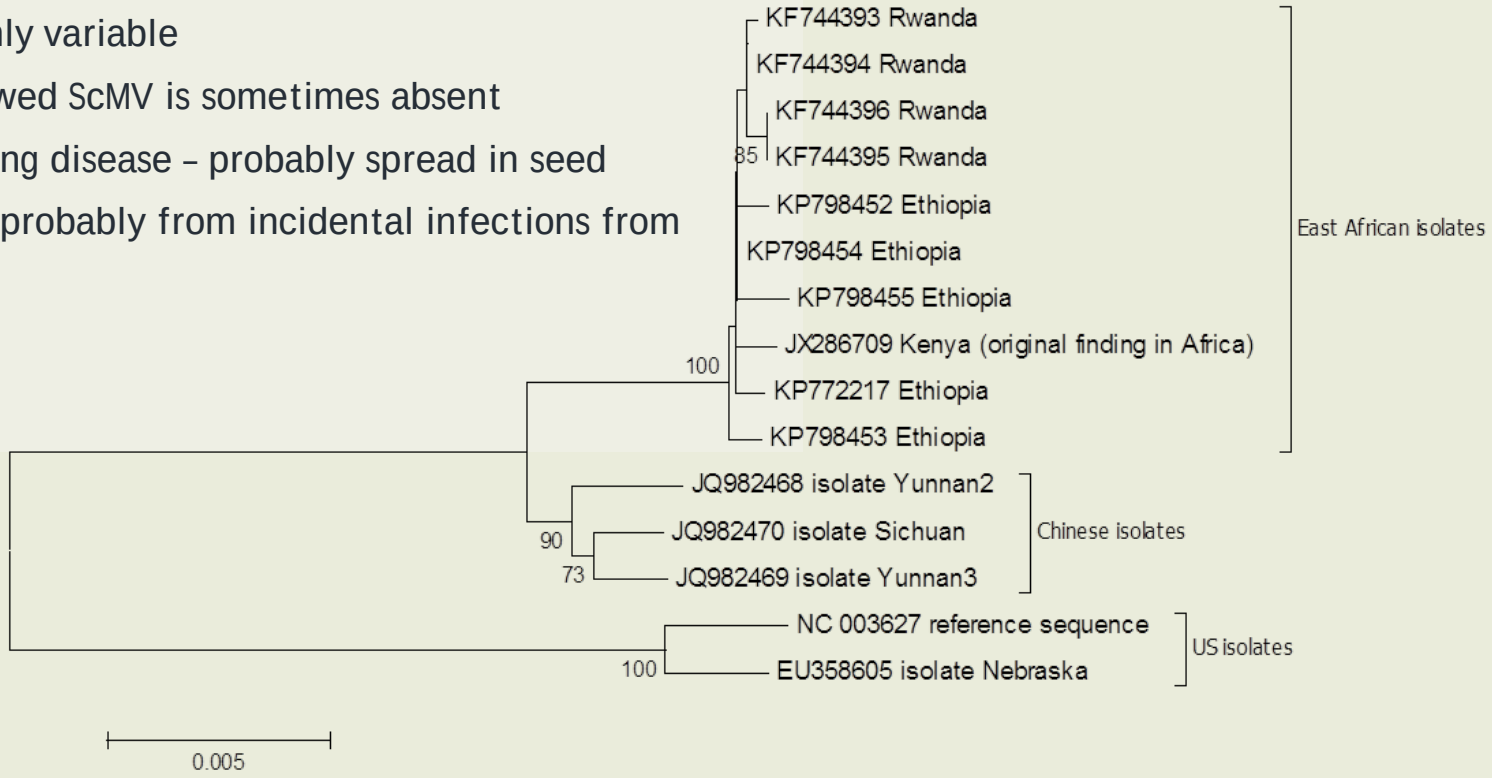
# Confirmed the cause

- Back testing confirmed the presence of two viruses
- Satisfied Kochs postulates by mechanical inoculation
- Maize lethal necrosis
  - Maize chlorotic mottle virus (MCMoV)
  - Sugarcane mosaic virus (ScMV)
- Helped identification of likely vectors
- Developed a seed test and trained Kephis/Kari staff

Adams, I. P, Mumford, R. & Boonham, N. 2013. Use of next-generation sequencing for the identification and characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis in Kenya. *Plant Pathology,* 62**,** 741-749

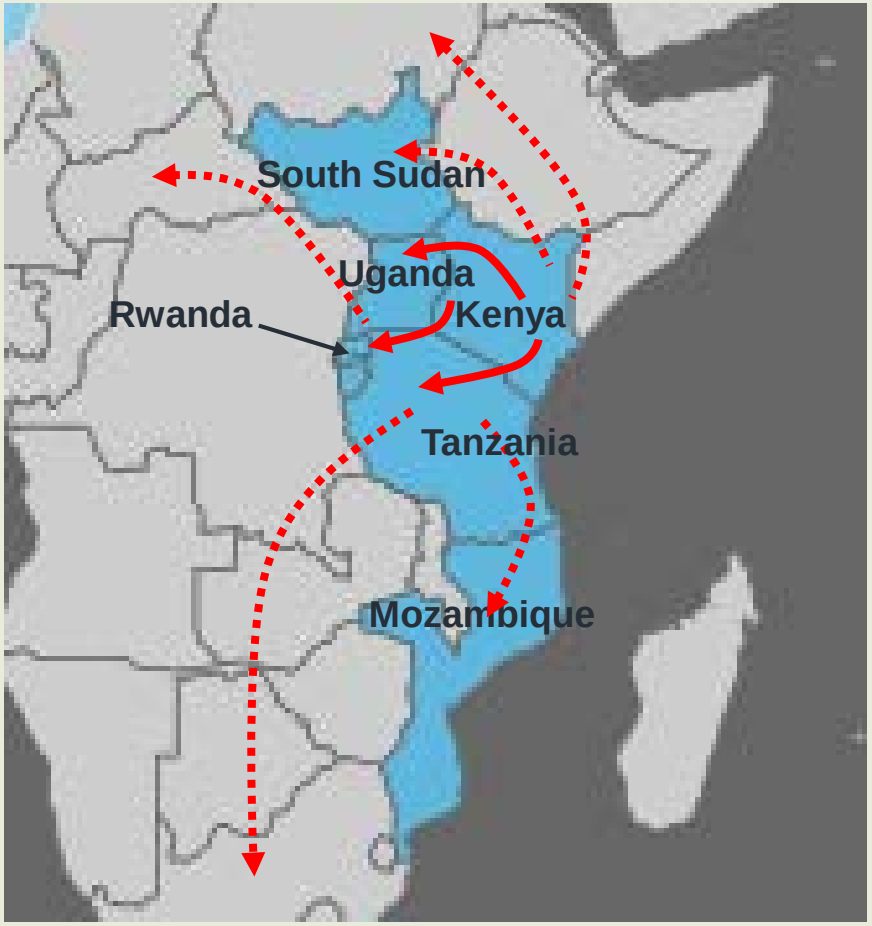
# Tracking the disease

- MCMoV is highly conserved
- ScMV is highly variable
- Testing showed ScMV is sometimes absent
- MCMov driving disease – probably spread in seed
- Potyviruses probably from incidental infections from weeds




Adams, I. P., Harju, V. A., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M., Smith, J., Fox, A., Uzayisenga, B., Ngaboyisonga, C., Uwumukiza, B., Rutikanga, A., Rutherford, M., Ricthis, B., Phiri, N. & Boonham, N. 2014. First report of maize lethal necrosis disease in Rwanda. New Disease Reports, 29.

# Disease spread



# Confusion with other diseases

- *Sugarcane mosaic virus* not always present in MLN plants
- *Maize yellow mosaic virus* commonly found

| Independent site | *Maize chlorotic mottle virus* | *Sugarcane mosaic virus* | Other *Potyvirus* | *Maize yellow stripe virus* | *Maize streak virus* | Maize yellow mosaic | Field Diagnosis | Lab Diagnosis |
|---|---|---|---|---|---|---|---|---|
| Kenya | Present | Present | Present | | | Present | MLN | MLN |
| Kenya | Present | | | | | | MLN | |
| Kenya | Present | | | | | Present | MLN | |
| Kenya | Present | | | | | Present | MLN | |
| Ethiopia | Present | Present | | Present | | Present | MLN | MLN |
| Ethiopia | Present | Present | | | | Present | MLN | MLN |
| Ethiopia | Present | Present | | | | Present | MLN | MLN |
| Ethiopia | Present | Present | | | | | MLN | MLN |
| South Sudan | | | | | Present | Present | MLN | |
| Rwanda | Present | Present | | | | | MLN | MLN |

# Deconstruct the maize virome

| | Crop | samples | known plant viruses | Novel plant viruses | total samples |
|---|---|---|---|---|---|
| Farm 1 | maize | 4 | Maize chlortic mottle virus, Sugarcane mosaic virus, Maize yellow dwarf mosaic virus | Tombusvirus, Carmovirus, Foveavirus, Closterovirus, betaflexivirus, positive strand ssRNA virus | 30 |
| | others | 26 | bean comon mosaic virus, Beet pseudoyellows virus, Maize yellow dwarf mosaic virus, SCMV, Potato virus S | Caulumoviridae virus, Chrysovirus,Crinivirus, Potyvirus(es), Tombusvirus, unclassified ssRNA positive strand virus, Varicosavirus, Filoviridae virus | |
| Farm 2 | maize | 9 | Maize chlortic mottle virus | chrystovirus luteovirus ,Carmovirus, tombusvirus, virus, positive strand ssRNA virus, unclassified virus | 29 |
| | others | 20 | Shallot latent virus, Cauliflower mosaic virus, | Chrysovirus, Crinivirus, Cytorhabdovirus, Waikavirus, Varicosavirus polerovirus, polerovirus associated RNA, Tymoviradae virus, positive strand ssRNA virus | |
| Farm 3 | maize | 6 | Maize chlortic mottle virus, Maize yellow dwarf mosaic virus | Badnavirus, polerovirus associated RNA, Tymoviradae virus | 26 |
| | others | 20 | Turnip mosaic virus, | Badnavirus, Chrysovirus, Cytorhabdovirus, unclassified ssRNA positive strand virus | |
| Farm 4 | maize | 4 | Maize chlortic mottle virus, Maize yellow dwarf mosaic virus | none | 30 |
| | others | 26 | Banana streak virus, Apple stem grooving virus, Citrus tristeza virus | Badnavirus, potyvirus(es), Tombusvirus, Rhabdoviridae virus, positive strand ssRNA virus, unclassified virus, Varicosavirus | |
| | | | | total | 115 |

# Nanopore sequencing

- Inexpensive sequencer
- Long read lengths
- Cloud based analysis
- Potential in resource poor labs where a large sequencer is impractical/expensive

# MinION against MiSeq

- Sequencing errors causing problems for *de-novo* detection of new viruses with BLASTx

| Analysis | MiSeq | MinION |
|---|---|---|
| BLASTn/x | 1 known, **4 novel** | 1 know, **2 novel** |

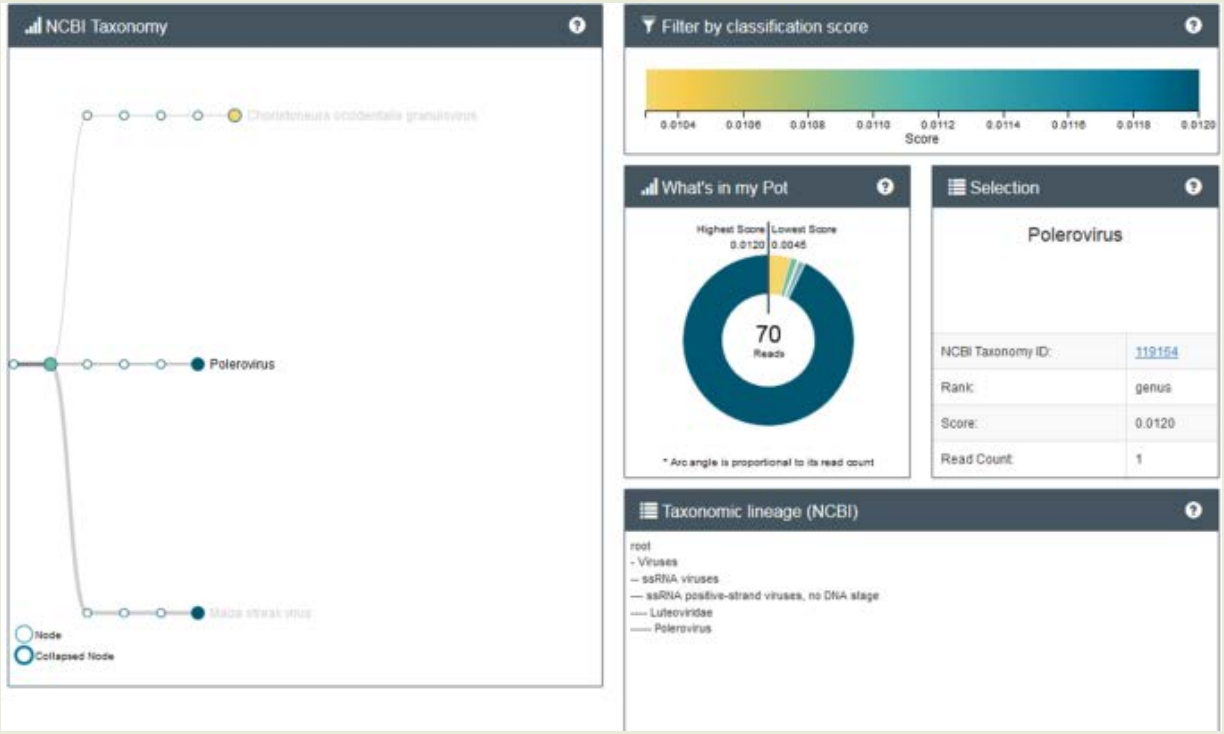
# Reference mapping

- Virus sequences were all present in the MinIon data

| Virus Sequence | RPKM MiSeq (BWA) | MinION (LAST) | |
|---|---|---|---|
| Maize Yellow Streak virus | 7615 | 11289 | Known maize virus |
| Polerovirus | 347 | 381 | Novel, related to Maize yellow dwarf virus |
| unclassified virus | 1220 | 735 | Novel virus |
| dsRNA virus RNA1 | 2847 | 656 | Novel virus |
| dsRNA virus RNA2 | 24280 | 5943 | |
| Totivirus RNA1 | 2619 | 2056 | Novel virus |
| Totivirus RNA2 | 146 | 375 | |
| Totivirus RNA3 | 211 | 291 | |

# What's in my pot ?

- Simple analysis pipelines available (WIMP)
- Identified one known and one new virus in samples from Ethiopia



# Future platforms

- Smaller – SmidgeIon
- Larger - PromethIon


# Automated solutions

- Sample prep – Zumbador
- Library set up - Voltrax

fera

## Collaborators

biosciences eastern and central africa

cabi.org

RWANDA RAB AGRICULTURE BOARD

KARI

KEPHIS

Oxford NANOPORE Technologies

## Funders

DFID Department for International Development

SWEDEN

syngenta foundation for sustainable agriculture