Evaluation of transgenic cassava with resistance to cassava viruses in confined field trials under the VIRCA project

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WHAT IS VIRCA

Virus Resistant Cassava for Africa (VIRCA) is a research project that is developing improved cassava varieties, which are resistant to cassava mosaic disease (CMD) and cassava brown streak disease (CBSD)
Cassava – strategic crop for Africa

- **Foliage**
  - Human food
  - Livestock feed

- **Stem**
  - Planting material

- **Roots**
  - Staple food
  - Unique starches

- Major source of food and income
- 2nd most important food crop in Africa after maize
- Strategic crop for increasing food supply, reducing hunger and responding to food emergency crises
Disease constraints

1. **Cassava mosaic disease (CMD)** has been the main disease constraint

1st reported 1894 in present day Tanzania

Caused an epidemic in the 1990s

Africa losses an average 30-40% (15-24 million tonnes; $6-25 billion/year)
Genome Organization and Replication of a Geminivirus

Geminate particles

8 Genes
Cassava-infecting begomoviruses (CMD)

- 9 distinct species, 7 in Africa
- ACMV and EACMV types most prevalent in EA

**Africa**

- East African cassava mosaic virus cluster
  - African cassava mosaic virus (ACMV)
  - East African cassava mosaic virus (EACMV)
  - East African cassava mosaic Malawi virus (EACMMV)
  - East African cassava mosaic Cameroon virus (EACMCV)
  - East African cassava mosaic Zanzibar virus (EACMZV)
  - East African cassava mosaic Kenya virus (EACMKV)
  - South African cassava mosaic virus (SACMV)

**India**

- Indian cassava mosaic virus (ICMV)
- Sri Lankan cassava mosaic virus (SLCMV)
Necrotic Rot of Storage Roots due to CBSD

- Complete spoilage, yield reduction
- Two viruses: CBSV, UCBSV

2. CBSD

- One of 7 most dangerous crops diseases in the world impacting food security (Science 327 – 12 February 2010)

- Similar features by New York Times and FAO
Genome Organization of aPotyvirus
Potyviridae: CBSD Viral pathogens

Only two genetically distinct virus species?
Whitefly, the vector of viruses causing CMD and CBSD
Challenge to management of the two diseases

- Continuous presence of the virus and the vector throughout the year
- Attachment of farmers to particular cultivars which are susceptible to the disease
- Limited number of resistant/tolerant genotypes preferred by farmers

VIRCA project was initiated to combat these virus problems
Vision: Food security for 30 million food insecure Ugandans and Kenyans who depend on cassava as their staple food.

Mission: Improve cassava harvests of smallholder cassava farmers in Kenya and Uganda by delivering genetically enhanced, farmer-preferred cassava varieties that can resist the Cassava Brown Streak Disease and the Cassava Mosaic Disease.
Virus resistant cassava for Africa (VIRCA) Project

Goals

Deliver farmer-preferred transgenic cassava resistant to CMD and CBSD to farmers in East Africa

Capacity building (human and institutional)
Training of staff – both locally and internationally

- Biosafety compliance and CFT management
- Molecular techniques, plant virology, data management
Improved facilities and equipments

- Construction of greenhouses, laboratories and CFT sites
The Technology

- Different technologies tested

- RNAi technology (gene silencing) technology selected
  - Principle similar to immunization
    - *Nature does not allow dsRNA in living organisms*

- Sequences derived from viruses causing the diseases in East Africa
Gene Silencing

- Also called RNA interference (RNAi)

- Results in down-regulation of a gene at the RNA level

- Referred to as PTGS when it occurs post transcription

- PTGS is a natural anti-viral defense system that occurs in plants

- Can be triggered by transgenes, viruses and dsRNA molecules.

- Once triggered GS is maintained by diffusible messenger that mediates the propagation of *de novo* PTGS through the plant.
Gene construct for CMD resistance

All the constructs were initially made in pILTAB-0588 vector carrying the Cassava Vein Mosaic Virus & 35S promoters and NOS poly A sequence using Xba I & Kpn I and BamH I & BstB I restriction enzymes.
CMD in Greenhouse

- Transgenic cassava lines showing resistance to EACMV; the effect of RNA-silencing constructs targeting the virus rep gene

- Clone K201 92% homology to EACMV-UG, 80-100% infectivity, faster progression of symptoms
Gene construct for CBSD viruses

- Near full length coat protein (CP) genes of both virus were used to make the inverted repeat
### CMD entries evaluated in the CFT

<table>
<thead>
<tr>
<th>Entry</th>
<th>Line</th>
<th>Vector</th>
<th>Gene of interest</th>
<th>Comments</th>
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<td>pILTAB670</td>
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<td>Susceptible control</td>
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<td>TMS 30572</td>
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<td>Resistant control</td>
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</table>
Field trial Results

Non-transgenic Control

Transgenic plants
CMD trials Conclusions

• Target virus controlled effectively over multiple seasons

• Need for stacking to control all CMD viruses
Necrotic Rot of Storage Roots due to CBSD

- Complete spoilage, yield reduction
- Two viruses: CBSV, UCBSV

CBSD outbreaks reported in the highlands in mid 2000s

- One of 7 most dangerous crops diseases in the world impacting food security (*Science* 327 – 12 February 2010)
- Similar features by New York Times and FAO
Entries evaluated

<table>
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<tr>
<th>Entry</th>
<th>Line</th>
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<td>15</td>
<td>CV. 30572</td>
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</table>
Symptoms of CBSD at Harvest in Uganda

- 95% of roots of Line 1-718 were free of necrosis
- 90% of roots of WT 60444 control had severe necrosis

Best test line; No rotting of the storage roots

Control line without the gene; severe rotting of roots
Summary

CMD

• Control of targeted CMD-causing viruses achieved
• Stacking of genes for control of different species needed

CBSD

• Control of both UCBSV and CBSV achieved in TME204
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