Research & Development Division Clean Seed programme

- Started in 2009
 - Reduction in Import bill
 - Strengthen food security
 - Improve production
 - Make money/reduce poverty

Several steps are involved in the establishment of a *Clean Seed Program*:

- selection of parent plants from cultivars with ideal traits (disease resistant, high yielding capability, colour and shape)
- screening of the selected parent plants to detect possible pathogens of economic importance
- 3) retrieval of pathogen-free plants (nuclear stock) by meristem culture *in vitro* and or thermotherapy
- 4) screening of newly retrieved plants (nuclear stock)
- 5) horticultural evaluation of the healthy plants,
- 6) maintenance and mass propagation of healthy plants and seeds under protected conditions.

Numerous efforts were placed on screening plants for diseases (Diagnostic), selecting genotype and phenotype of ideal characteristics (Crop Research), Cleaning (Tissue culture) and Rapid multiplication of clean and idealistic planting materials (Propagation Techniques).

The Programme involves application of improved technologies and investigations into the production of clean certified planting material for farmers to facilitate the growth and development of key industries. The Division target crops such as Sweet Potato, Irish Potato, Citrus, Ginger and Sweet Yam.

Retrieval of Pathogen-free Plants

- Heat Therapy
- Tissue Culture

-Meristem Culture

-Shoot Tip Grafting

Tissue Culture



Collection of propagative material for tissue culture



Meristem



Sweet Potatoes at 8 weeks in culture



Initiated Jamaica Yellow Ginger



Irish Potato microtubers

Screening/ Diagnostic Testing

Bio-indexing:



Grafting test plant onto another plant that is susceptible to the tested disease

Newly indexed plants

Conventional Method – Agar Plating, Microbe isolation and microscopy view ۲



Showing Drechslera spores

Showing Cucumber mosaic virus inclusion bodies

- Serology
 - Diagnostic Kits
 - ELISA (Enzyme-Linked Immunosorbent Assay)
 - DotBlot and Tissueblot Testing Nitrocellulose Membrane (NCM)
 - o Immunostrips



TissueBlot Testing – NCM ELISA



Citrus Tristeza Virus indicated by yellow



Serology – NCM membranes showing dark purple positives. Brown color due to possible tannins and other biomolecules in sap

- Molecular DNA/RNA
 - Conventional PCR (Polymerase Chain Reaction)
 - o Real Time PCR

Figures showing stages in PCR testing



1) Discs are removed from sample leaves and crushed in buffer. 2) Resulting sap suspension is collected and DNA extracted using a modification of the Dellaporta method or extraction kits. 3) Total DNA (plant and pathogen) is precipitated out of solution and allowed to 4) air dry before dissolving in sterile water for PCR and storage.



Above figure showing gels run on 2% agarose after PCR for HLB. In all gels Lane 1 is the DNA ladder, lane 2 is the positive control and lane 3 is the negative control. Lane 10 in gel 3 is a sample from the field that was positive.

Maintenance and Mass Propagation under Protected Conditions

Newly established planting materials from the tissue culture facility are housed in protective greenhouses of various kinds, from low technology to high technology

High Technology





Low Technology



Production of 1st generation

Irish Potatoes



Sweet Potatoes





Ginger



