

GENETICALLY MODIFIED BANANA FOR RESISTANCE TO BLACK SIGATOKA DISEASE

Experience with a confined field trial

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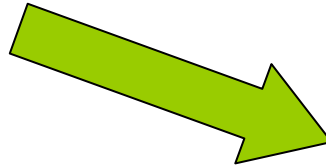
Background

- Banana important staple for millions in Uganda
 - Production: 10 million tons/year
 - Consumption: 243 kg/cap/year
- Grown by mainly resource limited farmers

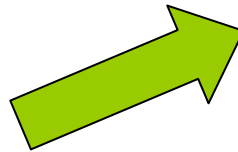


Pests

weevil



nematodes



Diseases

Black sigatoka (BSD)



Banana bacterial wilt (BBW)



Breeding for host resistant to diseases and pests

– Conventional breeding - ongoing

- Development of matooke hybrids
- Development of improved male parents
- Limitations:
 - Sterility
 - Polyploidy
 - Complex genetics
 - Long generation time

– Biotechnology

- Gene identification
- Genetic engineering

Banana genetic engineering

Acquiring capacity in biotechnology:

- Trained personnel
- Infrastructure

Banana biotechnologies developed:

- Cell suspension system developed
- Transformation system developed
- Genes for key constraints acquired
- Transgenic bananas developed and evaluated

Capacity building in Biotechnology

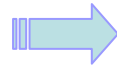
- Infrastructure
- Personnel



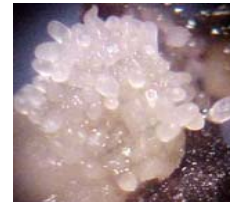
Banana Transformation System Developed



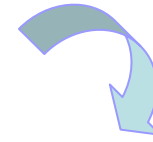
Male bud



Immature flower

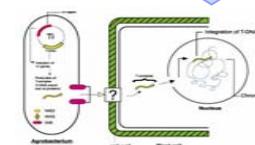


Embryogenic callus



ECSs

ECS initiation and transformation



ECS infection



Selection



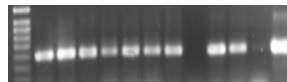
Shoots from EC clones



Morphological analyses



PCR analyses

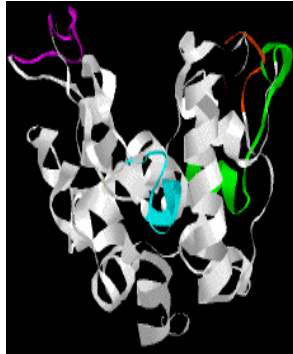


Southern blot analyses



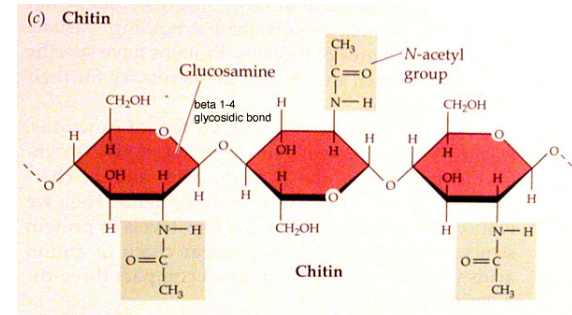
Strategy for BSD: Plant Chitinase

Chitinases



Degradation

Chitin



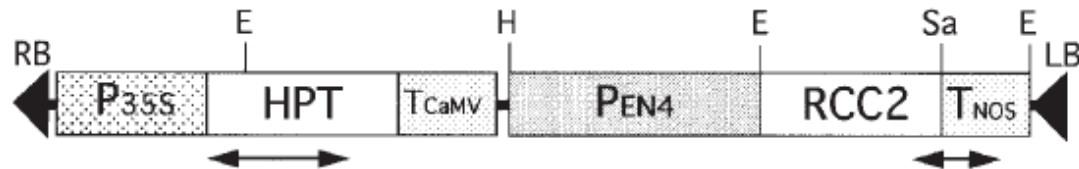
Plant defense responses

Fungal cell walls

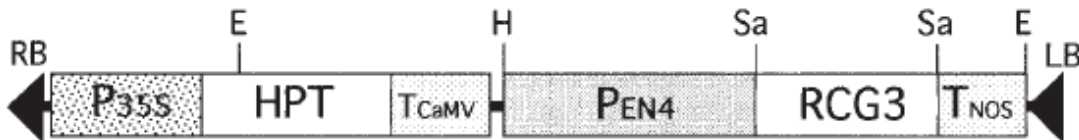
- Restrict fungal growth
- Reduce Disease development
- Confer increased resistance in transgenic plants
- Rice chitinase *rcc2* (from mRNA) & *rcg3* (from genomic DNA) were cloned from rice (Nishizawa *et al.*, (1993)
 - Rice chitinase : cucumber (Kishimoto *et al.*, 2002)
 - Rice chitinase : italian ryegrass (Takahashi *et al.*, 2005)

Gene constructs

<< pBI333-EN4-RCC2 >>



<< pBI333-EN4-RCG3 >>



-**P35S** : Cauliflower Mosaic virus (CaMV) 35S promoter

-**HPT**: Hygromycin phosphotransferase encoding gene

-**PEN4**: Enhanced CaMV 35S promoter

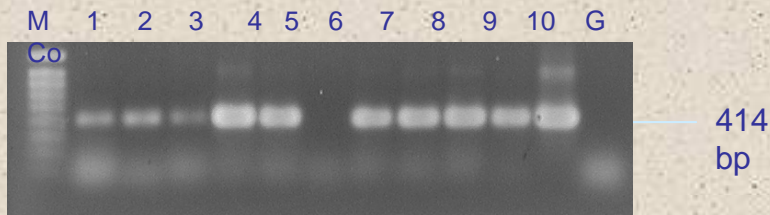
-**RCC2**: Coding region of Chitinase-2 gene
+ signal peptide for vacuolar targeting

-**RCG3** : Coding region of Chitinase-3 gene
+ signal peptide for secretion

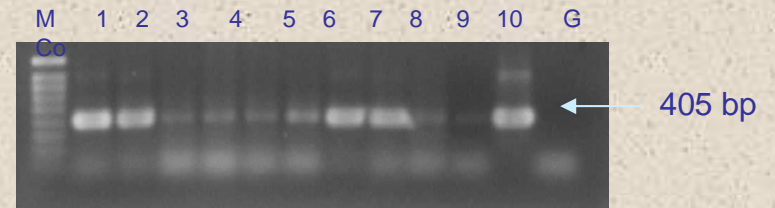
Both *rcc2* and *rcg3* were integrated into banana cultivar Gros Michel (AAA)

PCR analysis for chitinase genes

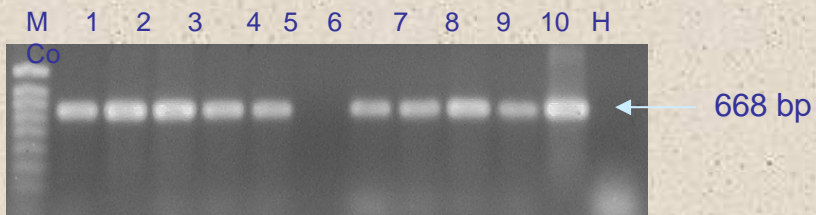
RCG3



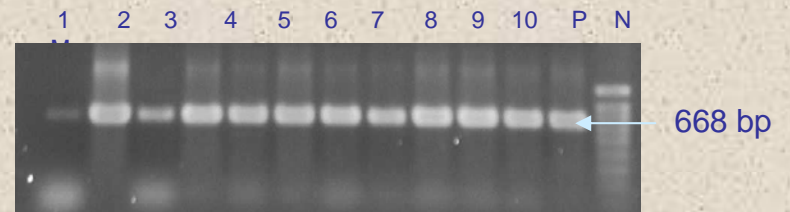
RCC2



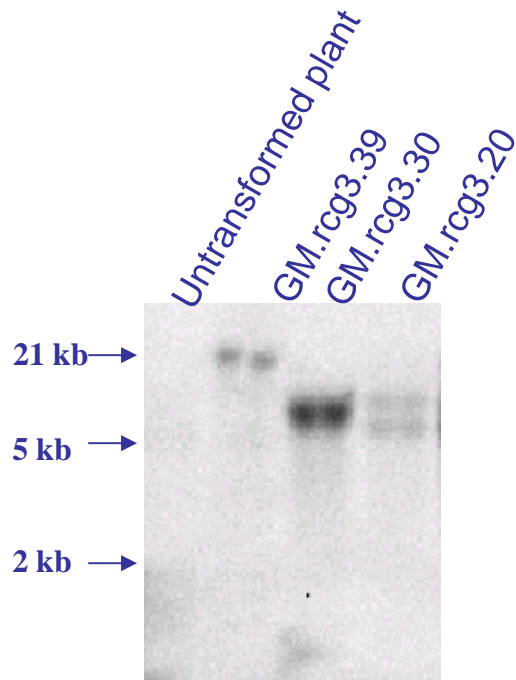
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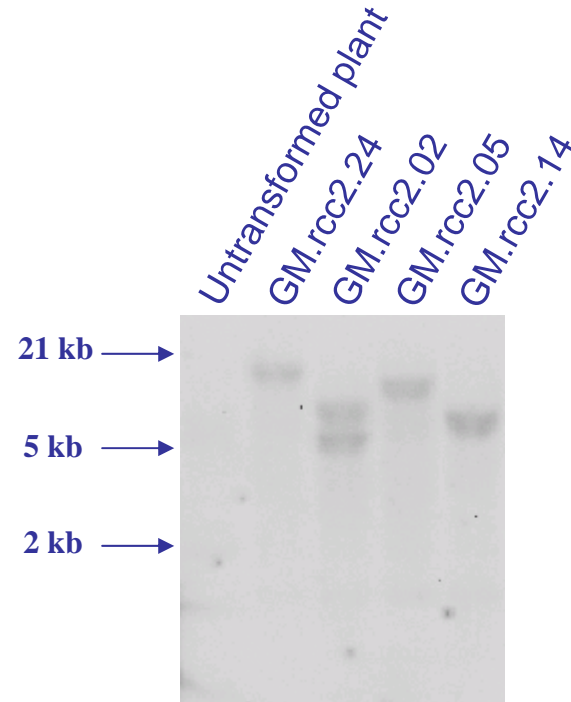
hpt



Preliminary Southern analyses for chitinase genes



rcg3



rcc2

Ten lines of *rcc2* transgene and 25 lines for the *rcg3* transgene have been selected for field testing

CFT Rational

- Bioassays frequently used in BSD resistance development
 - Not consistent
 - Characteristic *M. fijiensis* infection and sporulation are not observed *in vitro* or in the greenhouse
- Field *M. fijiensis* infection, BSD development, give natural and correct BSD pressure
- CFT should hence show correctly resistance as provided by integrated and expressed transgene

Objectives of the trial

- Determine extent of transgene expression in the field
- Analyse efficacy of transgenes for resistance to BSD in the field
- Determine field testing procedures for banana GMO
- Capacity building in transgenic field trial experimentation at NARO
- Other biosafety studies

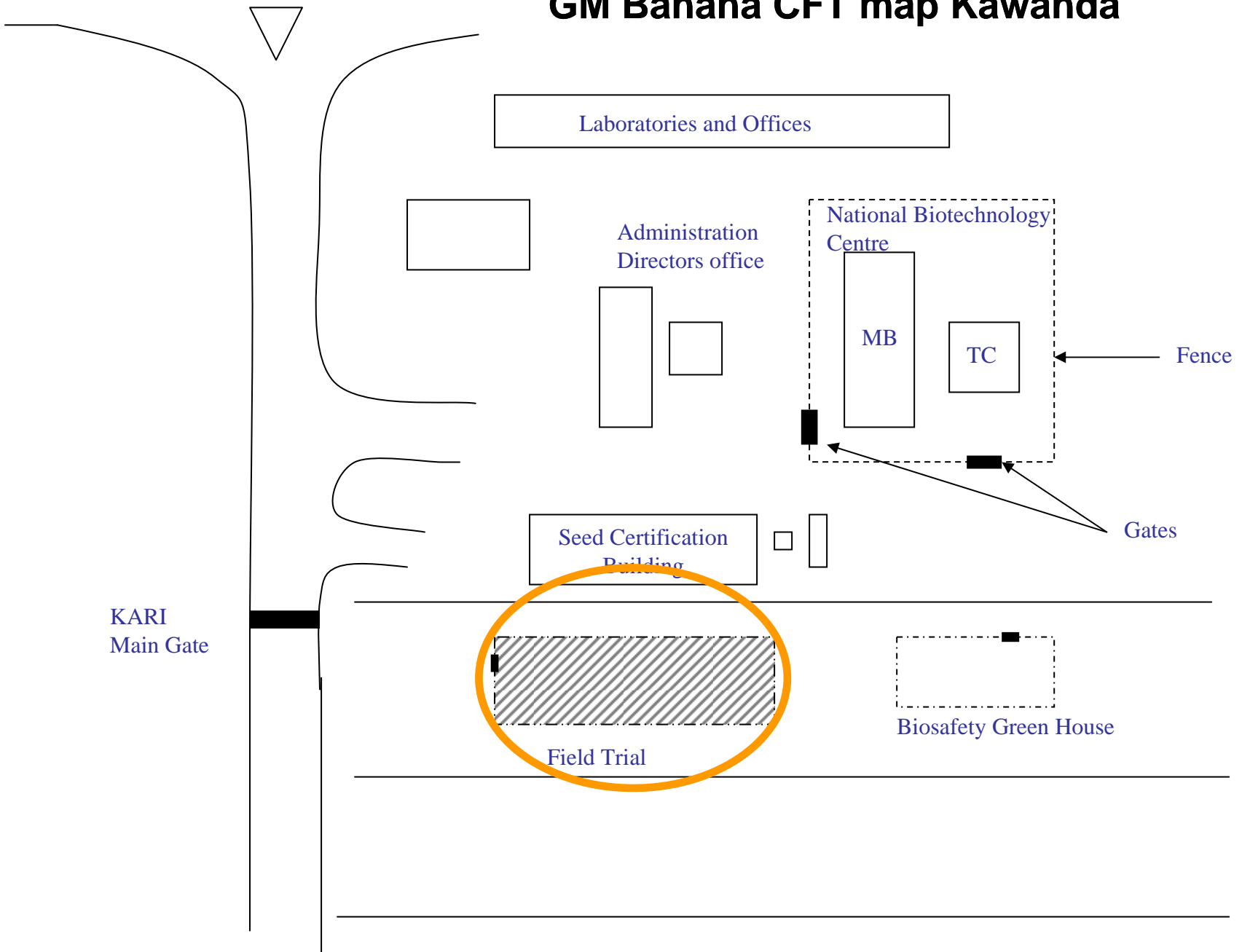
Confined Field Trial

Type		Quantity		Origin	Total number of plants
Trans-genic	Construct 1	10 events	9 plants per event	KUL	90
	Construct 2	25 events	9 plants per event	KUL	225
Non trans-genic	Control regenerants	5 regenerants	9 plants per regenerant	KUL	45
	Control	1 line	9 plants	NARO	9
TOTAL					369

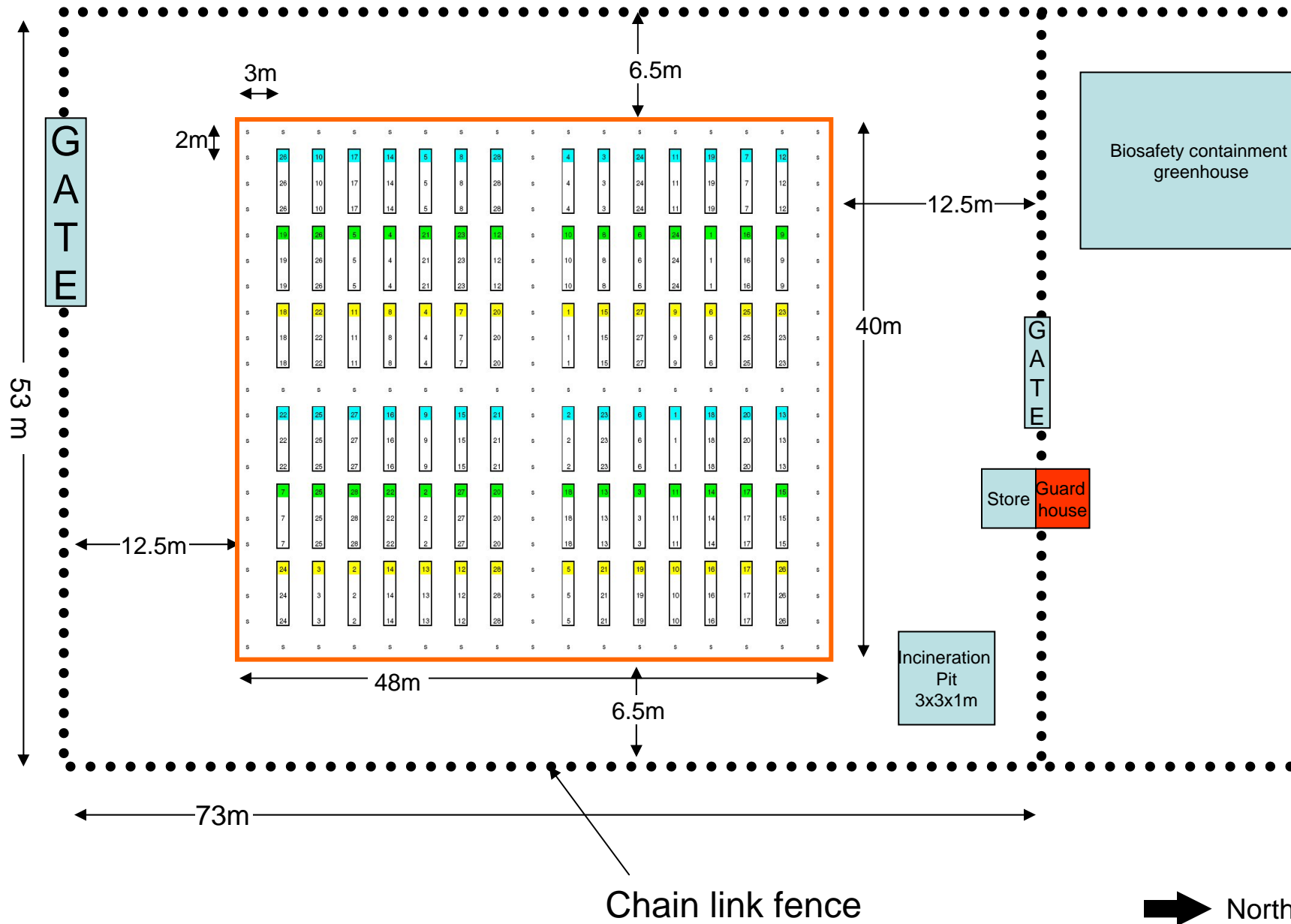
CFT Location

- Site
 - National Agricultural Research Laboratories
- Size of Introduction
 - 420 Transgenic banana tissue culture plants
- Land Area
 - 0.5 hectares of land
 - 0.25 hectares for the experimental trial
- Choice of location
 - Institute mandated for banana research
 - Close to National Agricultural Biotechnology Centre

GM Banana CFT map Kawanda



GM Banana: CFT Field plan-Kawanda

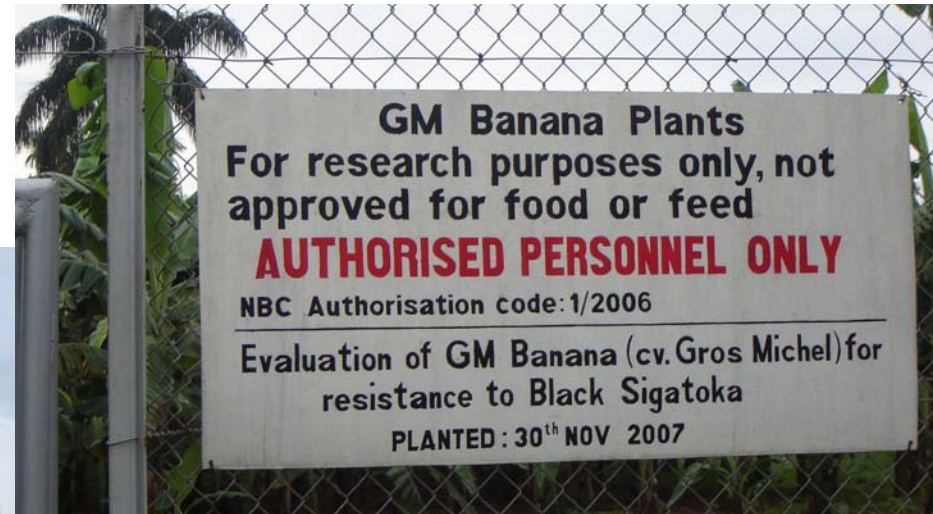


North

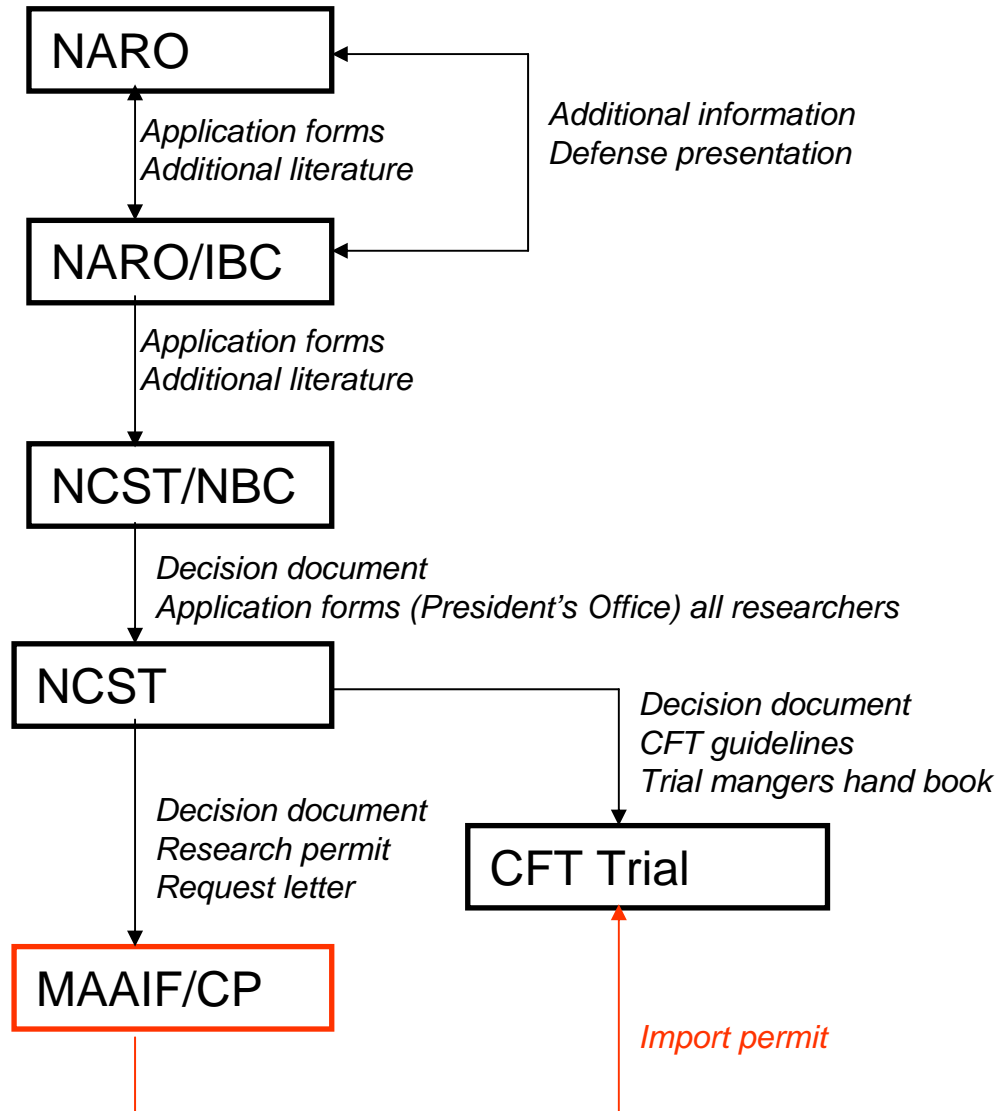
Plant inspection at Plant protection Department



GM Banana: CFT Planted Field



Regulatory process



Banana CFT: Timeline

- CFC application to IBC completed - Nov. 2005
- Approval issued by NBC - Aug 2006
- Research Permit issued - Oct.2006
- Import permit expected - July. 2007
- Plants arrived in Uganda - Aug. 2007
- Trial Establishment - Nov. 2007

Trial management cont'd

- **Data collection and Analysis**
 - Phenotypic characterisation
 - Black sigatoka diseases incidence
 - Record presence of symptoms of other diseases
 - Monitor transgene integration stability
 - Monitor expression of citinases

- **Management of residues and trial termination**
 - All residue to be incinerated
 - Bunches to be harvested before ripening and destroyed by incineration
 - At end of Trial, all plants to be treated with Glycosate and dead residue incinerated

Trial management

- **Handling**

- Special key and lock conditions to prevent un-wanted release of plants
- Trained staff (GMO biosafety); 24 hours guard

- **Transportation**

- Plantlets individually sealed in plastic bags
- Inspected by quarantine on arrival
- Stored in tissue culture laboratory Kawanda

- **Establishment**

- Hardening of plant in Plastic sheet tunnel inside greenhouse
- Any plants that die to be immediately destroyed
- Spacing 2m x 3m; randomised blocks of 3 plants each
- Inoculum spreaders used as borders rows

- **Maintenance**

- For a complete growth cycle – fruit production
- Standard banana trial management practice, e.g. pruning, application of manure, mulching – all residue incinerated

CFT's contribution

- Building a regulatory framework for CTFs in Uganda
- Building capacity at NARO, UNCST, MAAIF in handling GMO
- Public awareness of GMO technology

Thanks

