Development of Weevil Resistant Sweetpotato Varieties in Uganda using Biotechnology Tools

G. SSEMAKULA, A. SEFASI, R. RUKARWA, R. MWANGA, M. GHISLAIN & J. LOW

SWEETPOTATO ACTION FOR SECURITY AND HEALTH IN AFRICA

Background

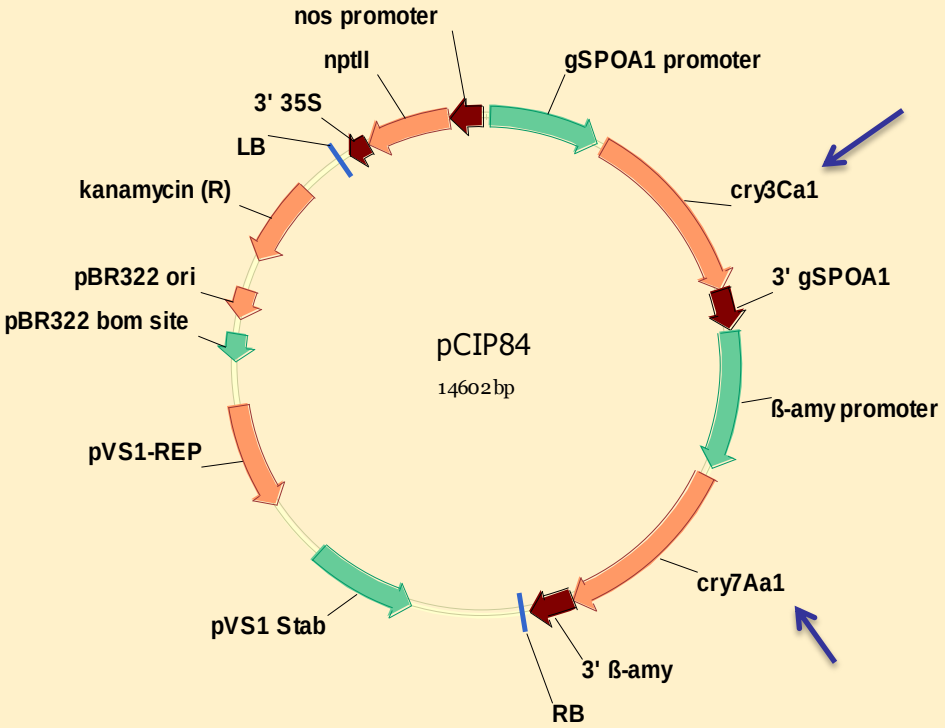
- Average yield loss due to weevil exceeds 28% in Uganda (Kiiza *et al., 2009)*
- IPM is difficult to implement
- Effective weevil resistance has not been found in available germplasm
- Bt crops have been safely used to control target pests

Crops on the Mkt with Bt genes

- Colorado potato beetle resistant potatoes with ***cry3A*** gene from *Bt* (subsp. Tenebrionis) released in 1996 by Monsanto.
- Corn rootworm resistant maize with ***cry3Bb1*** *gene from Bt subsp. Kumamotoensis* released in 2001 by Monsanto
- Corn rootworm-resistant maize with ***cry34Ab1*** and ***cry35Ab1*** genes from *Bt* strain PS149B1 released in 2005 by Dow.
- Corn rootworm resistant maize with a modified ***cry3A*** gene released in 2007 by Syngenta.

Approach to Developing WRSP in Uganda

Direct gene transfer: *Agrobacterium* mediated transformation with 2 Cry genes



Transgenic breeding: Crossing transgenic Jewel with Ugandan Cvs.

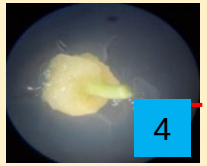
Direct gene transfer

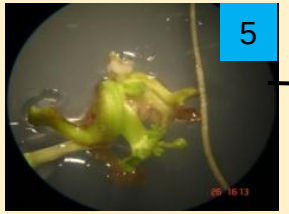
Optimizing the regeneration protocol

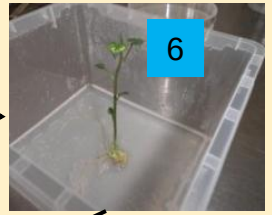
- Most genetic transformation methods require a tissue culture regeneration protocol
- Most African Cvs are recalcitrant to regeneration
- We are using the protocol of Kreuze *et al. (*2008) :12-15 months with CV Huachano with minor modifications

The regeneration protocol

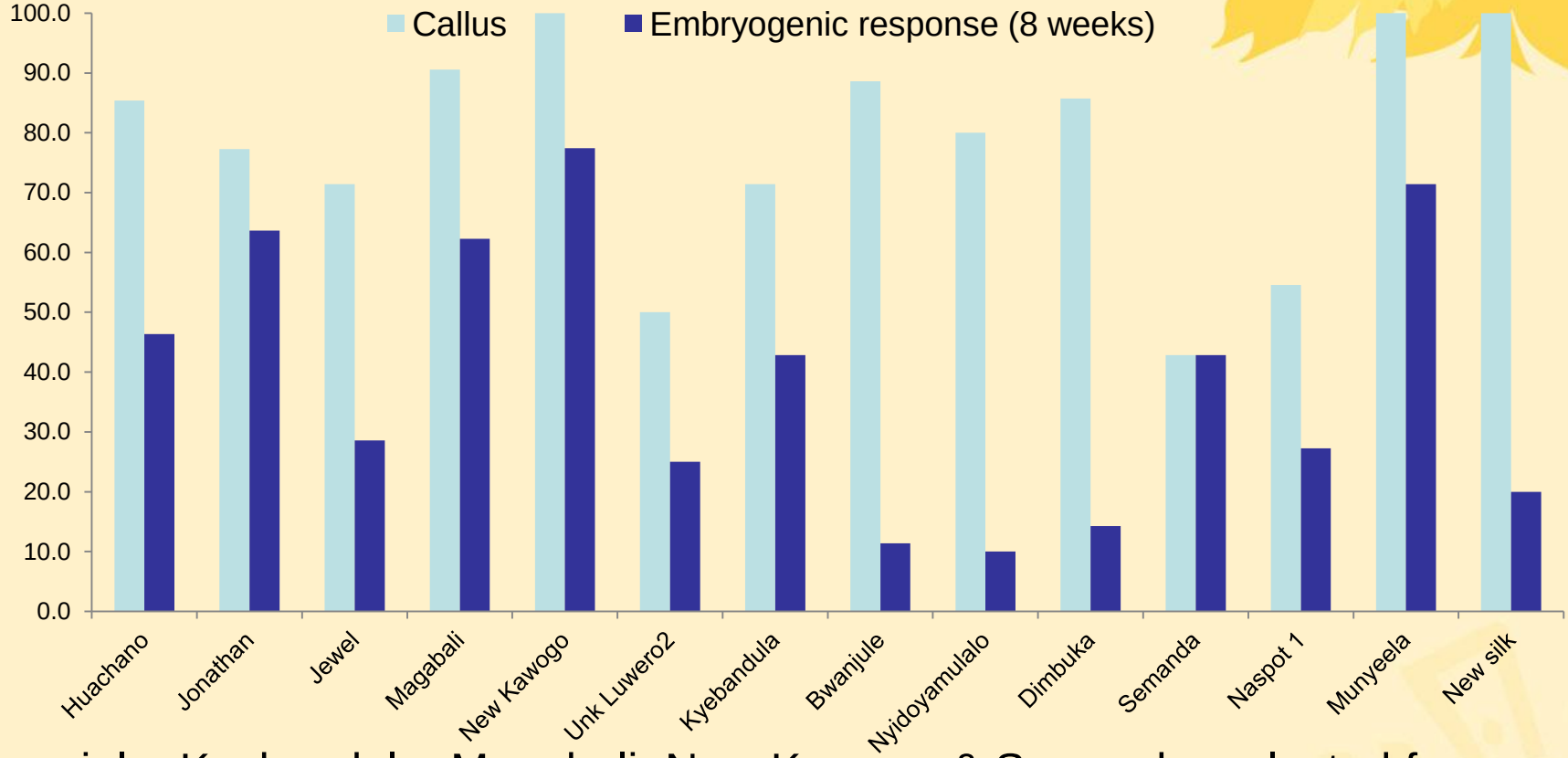
1. Ex-plant preparation
↓
2. Callus induction
↓ 3 d
3. Induction of embryonic structures
↓ 7 wks
4. Somatic embryos
↓ 3-5 mo
5. Shoot formation
↓ 1 mo
6. Rooting
↓ 3 mo
7. Multiplication







Popular Ugandan Cvs tested for ability to form embryogenic callus

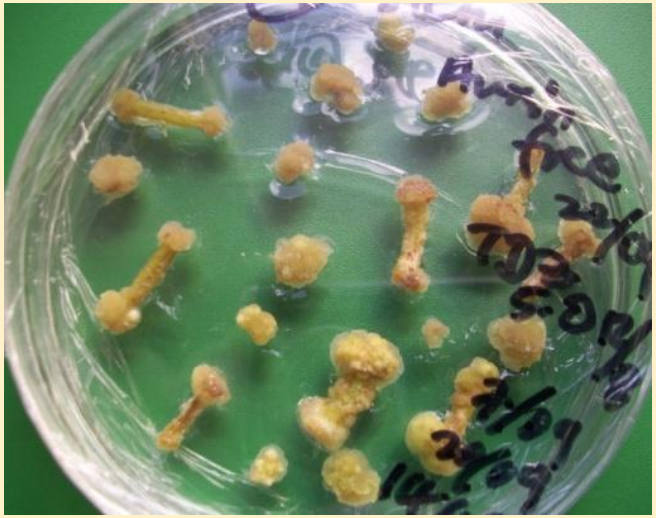


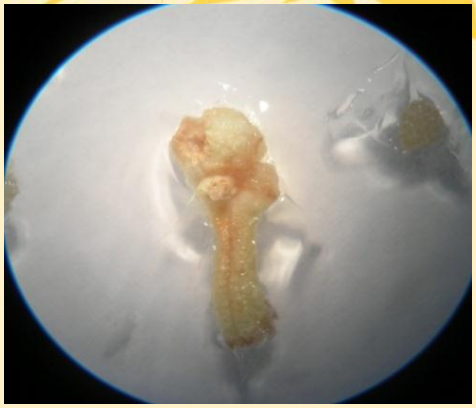
Bwanjule, Kyebandula, Magabali, New Kawogo & Semanda selected for transformation

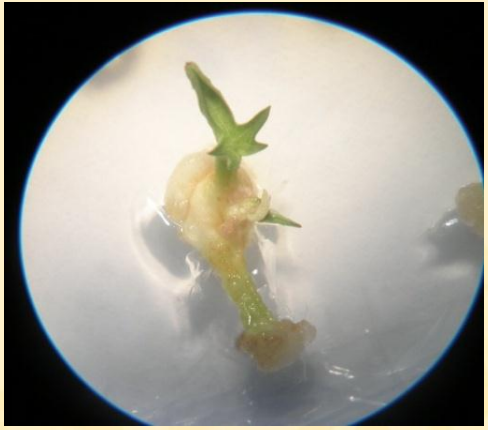
Regeneration cont.…

- The Cvs were subjected to somatic embryogenesis regeneration protocol (Kreuze *et al.* 2008) with minor adjustments
- Bwanjule and New Kawogo produced only roots
- The non-Ugandan Cvs (Jewel, Jonathan & Huachano) regenerated shoots & roots
- Both organogenesis & embryogenesis protocols are currently being used (emphasis on embryogenesis)

Regeneration through direct organogenesis







Bwanjule (5 plants) and Kyebandula (1 plant) were able to produce shoots through direct organogenesis on media with TDZ

Progress

Cultivar	Embryogenesis		Organogenesis		Total plants regenerated
	Callus (No.)	Plants regenerated (No.)	Explants (No.)	Plants regenerated (No.)	
Bwanjule	140	0	76	1	1
Kyebandula	651	0	81	13	13
Magabali	97	0	50	0	0
N/Kawogo	213	0	66	0	0
Semanda	141	0	46	0	0
Namusoga	N/A	N/A	241	0	0
Naspot 8	N/A	N/A	217	0	0
Munyeela	N/A	N/A	84	0	0
Araka red	N/A	N/A	216	0	0

- Embryogenic calli requires extra 1-3 months before regeneration

Way forward

- Putatively transformed plants will be screened (PCR and southern blot)

- Expression studies(RNA & protein) of the WR genes will be conducted

Transgenic breeding

Why transgenic breeding?

- Genetic transformation more successful with non-African varieties [Jewel, Huachano, Jonathan]

- Identification of a genotype with high accumulation of Cry proteins causing high mortality of SPW offers an opportunity for crossing with African germplasm

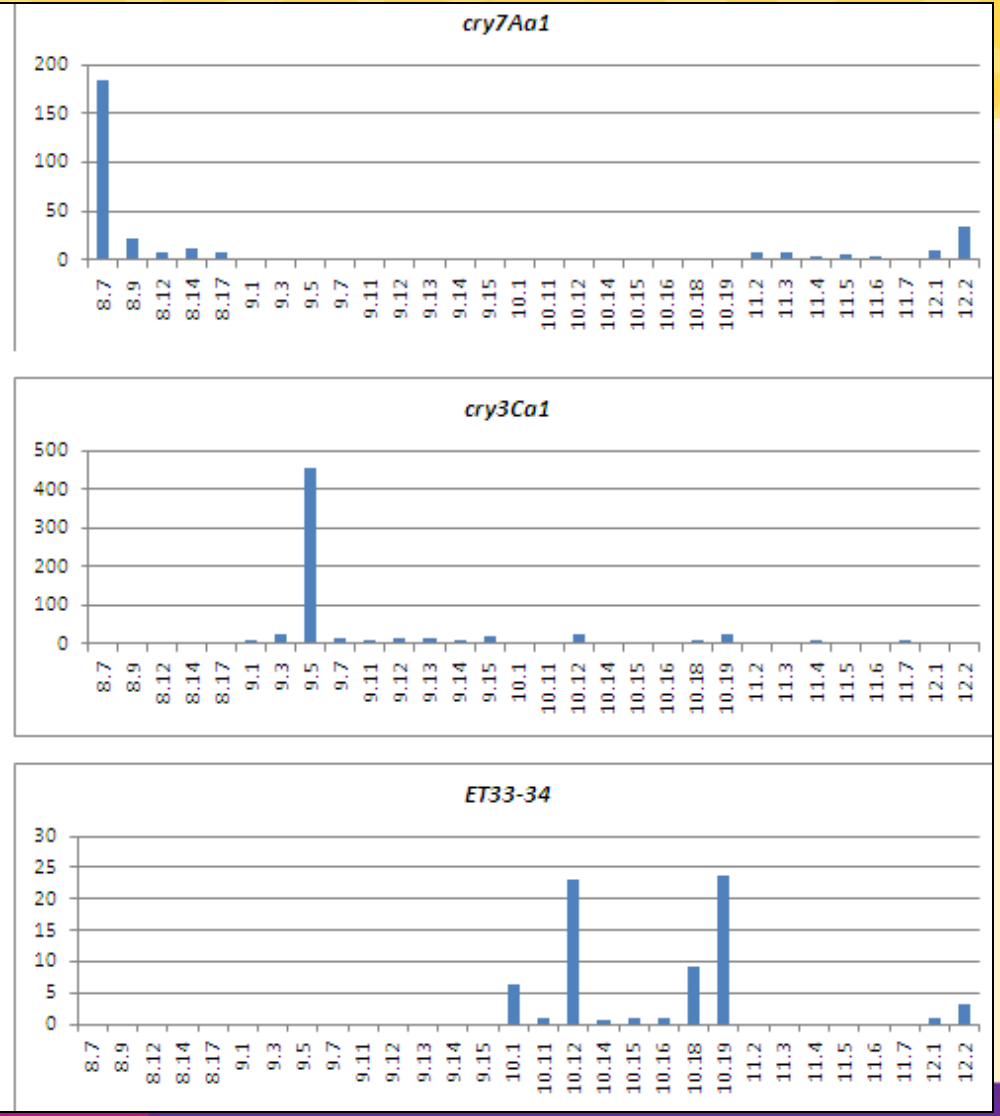
- Expectation: A SPWR Cv adapted to African agro-ecologies and for different end-uses.

Events introduced

- 33 transformed events from Jewel (extensively characterized at molecular level) from ABL-Lima

Number of events	Gene	Binary plasmid
8	***cry7Aa1***	pCIP78
9	***cry3Ca1***	pCIP79
8	***ET33-34***	pCIP82
6	***cry7Aa1 + cry3Ca1***	pCIP84
2	***cry7Aa1 + ET33-34***	pCIP85

Molecular characterization: QRT-PCR



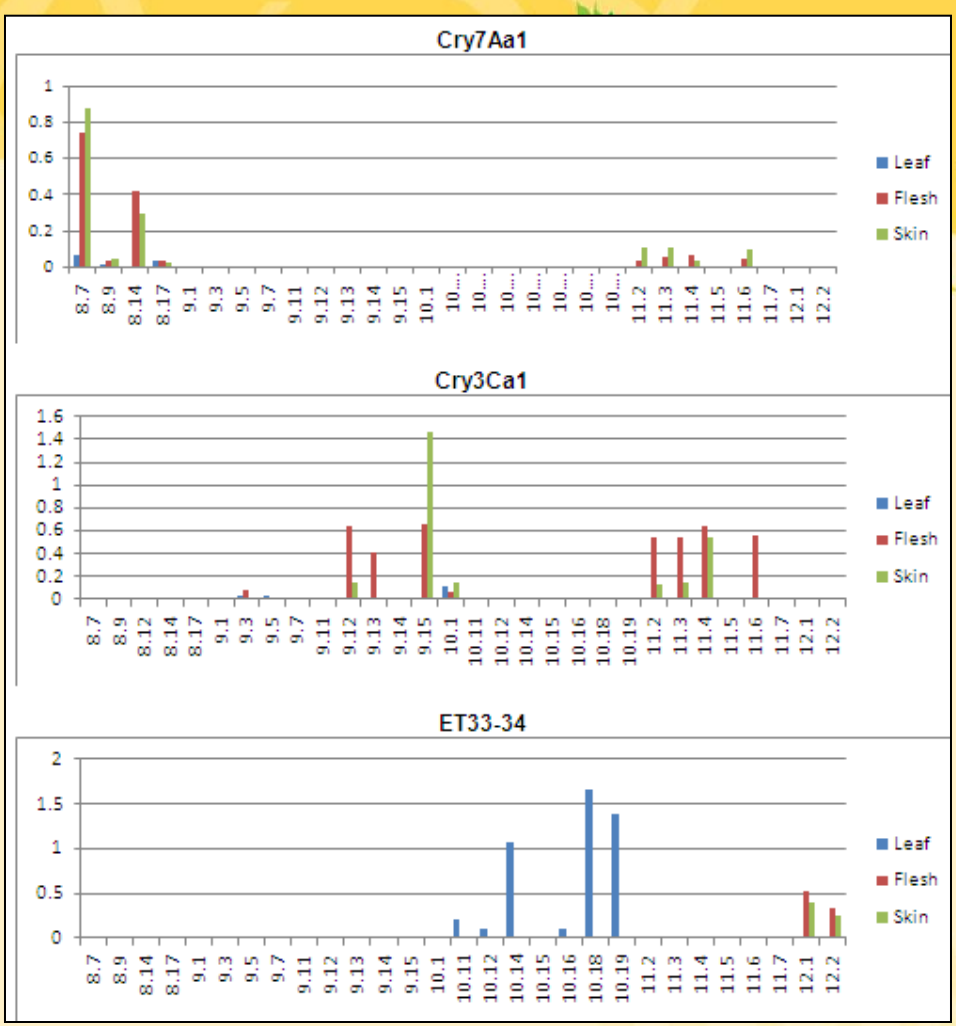
- Relative expression plot of SWR genes in leaves of transformed events from Jewel:
  - high expressers for each *cry* gene identified

- Y-axis = relative amount with respect to lowest expresser event.

- X-axis = events

Molecular characterization: ELISA

- Quantification of Cry protein in leaves, skin and storage roots flesh using DAS ELISA
- No correlation between transcription in leaves and Cry protein accumulation in storage roots
- Level of Cry Protein accumulation in the storage root flesh is inferior to LC50 (<1ppm) for some events



Y axis = protein (as mg/ g fresh weight .
X axis = events

Best candidates for WR

Best candidate events for conducting bio-assays & crossing with African cultivars identified:

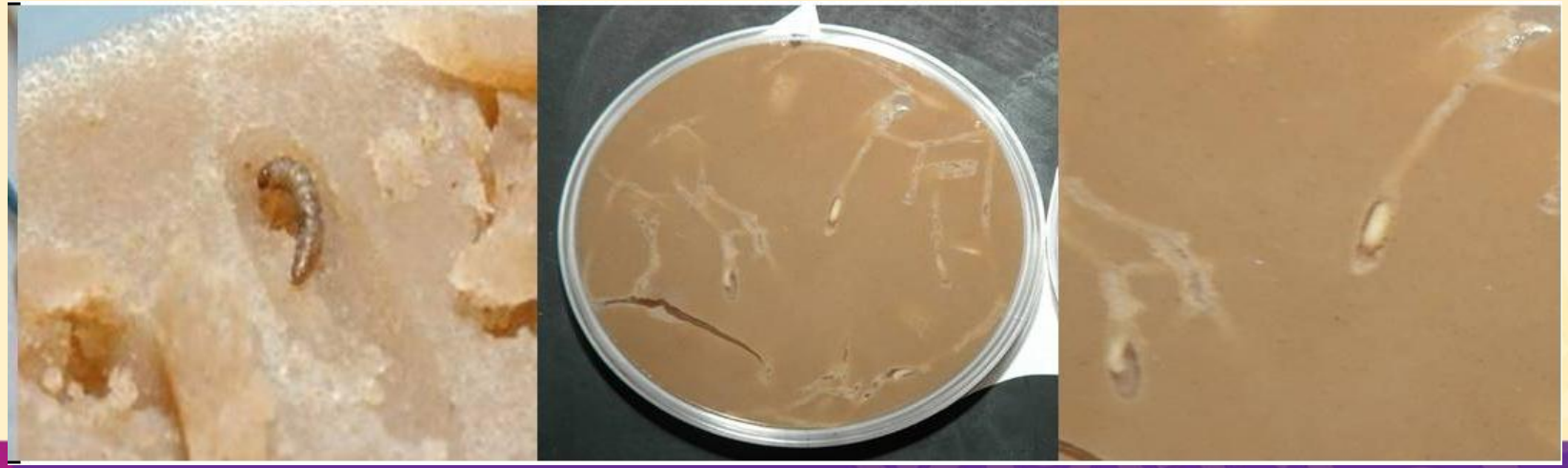
CIP 410008.7	cry7Aa1
CIP 410009.15	cry3Ca1
CIP 410010.19	ET33-34
CIP 410011.4	cry7Aa1 + cry3Ca1
CIP 410012.2	cry7Aa1 + ET33-34

Storage roots produced at BecA, KU & NaCRRI

Bioassays: Artificial diet

- Artificial diet with transgenic root powder (@ 8% wt/vol) infested with 2nd instar larvae of *C. puncticollis in petri dishes (10 larvae/ dish, 3 reps) for* 15d – control included
- high mortality in both transgenic & controls (suspect fungal infection as cause, ltd root powder)

– **To repeat expt, now that we have roots, we'll manage the fungus**



Bioassays: Whole root

- 10 female adults infested/root for 2d to lay eggs
- Roots incubated till adult emergence (3 reps)
- Expt repeated twice

- Contrasting resistance levels even within an event
- High variability in fecundity among females
- Differences between events not statistically significant

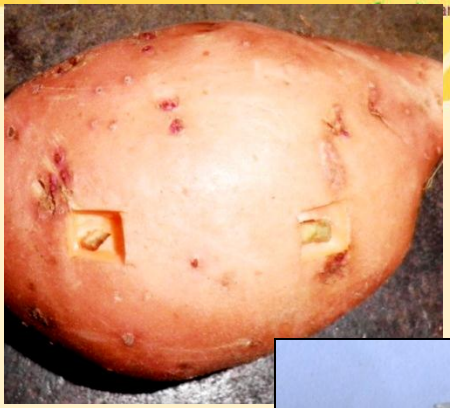
Bioassays: Root chips

- A piece of Naspot 1 with 2-24hr eggs plugged onto **transgenic Root chips** (4 reps)

- Difficult to manage fungal contamination up to weevil emergence
- Adults emerged from non-contaminated chips
- Protocol abandoned

Bioassays: whole Root with egg plug

- A piece of Naspot 1 with 5-24hr eggs plugged onto a whole **transgenic root** (5 reps)
- Control of non-transgenic jewel included
- Most eggs emerged into adults
  - no significant differences



Initiating process of conducting bioassays with purified Cry protein (non& truncated)

Gene introgression

- Events with high expression of Cry protein (CIP410008.7, CIP410009.15, CIP410010.19, CIP410011.4 and CIP410012.2) grafted to *I. setosa* to induce flowering.

- Crossing underway with New Kawogo, Tanzania and Naspot 1.
- More grafting in progress
- Event CIP41009.15 and local cvs not flowering in screen house; even with grafting

Gene introgression: progress

Family	Seeds
CIP410008.7 x Naspot 11	12
CIP410008.7 x N/Kawogo	10
CIP410008.7 x Tanzania	16
CIP410010.19 x Naspot11	4
CIP410011.4 x N/Kawogo	4
CIP410012.2 x N/Kawogo	9
CIP410012.2 x Naspot 11	9

Way foward

- **Selection of transgenic F1s: PCR analysis** using specific primers for *cry7*Aa1, *cry3Ca1* and *ET33-34* genes

- **Progenies with the transgenes**: evaluated for expression of the Cry protein thru DAS-ELISA

Bt = Big thanks

- CIP
- Makerere University
- NARO/NaCRRI
- NARO/NALRI
- RUFORUM
- University of Ghent (IPBO)
- BecA / ILRI
- Rockefeller Foundation

One day we shall get there!!!

Thank you