Progress towards development of WRSP Varieties using the Bt technology

SASHA

Sweetpotato Action for Security and Health in Africa

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on behalf of the WRSP team

Objectives of year 4

We wanted:

1. To achieve genetic transformation of African varieties
2. To evaluate different bioassays for testing transgenic events for efficacy
3. To quantify Cry protein accumulation in transgenic events from Jewel
4. To develop new *cry* gene constructs for enhanced accumulation

Genetic transformation update

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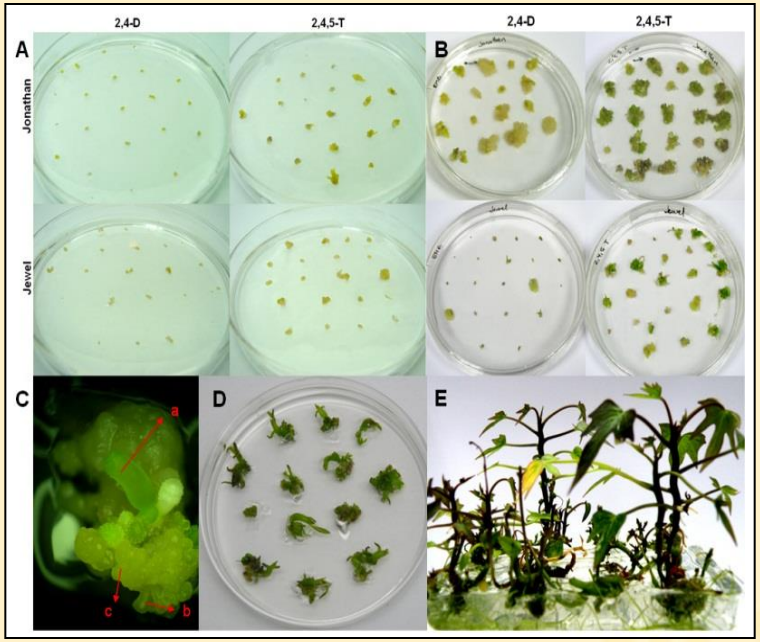
At ABL Peru:

- Somatic embryogenesis works best with Imby and Jonathan
- Protocol improvements following visit to Korean and Chinese labs (liquid cultures of somatic embryos)
- Transferred 33 transgenic events (TE) from Imby, and 31 other varieties with pCIP 85 (*cry7Aa1* and *cry3Ca1* genes)

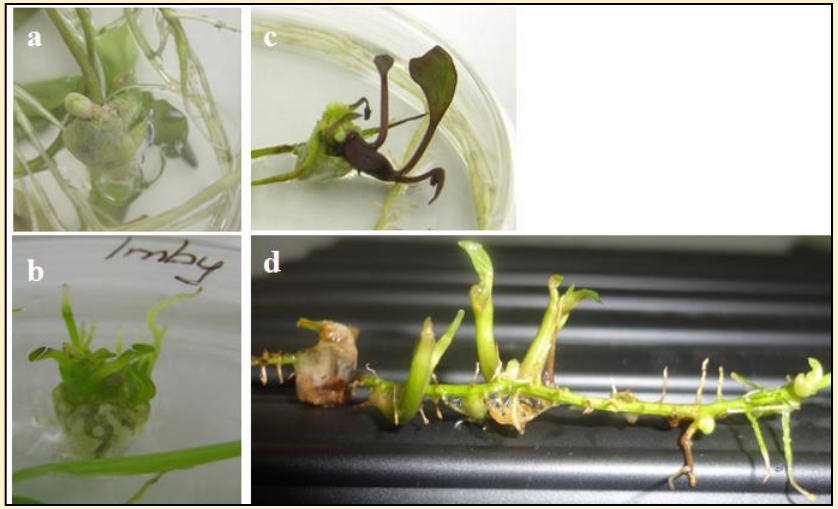


Manrique-Trujillo et al., 2013. Sweetpotato plant regeneration via an improved somatic embryogenesis protocol. Scientia Horticulturae, 161: 95–100

Genetic transformation update

At BecA Kenya:

- Organogenesis protocol works best with Ukerewe while somatic embryogenesis works best with Kagamega 7
- Produced 3 and 11 TE from Luapula and Ukerewe with pCIP85 (*cry7Aa1* and *cry3Ca1* genes)

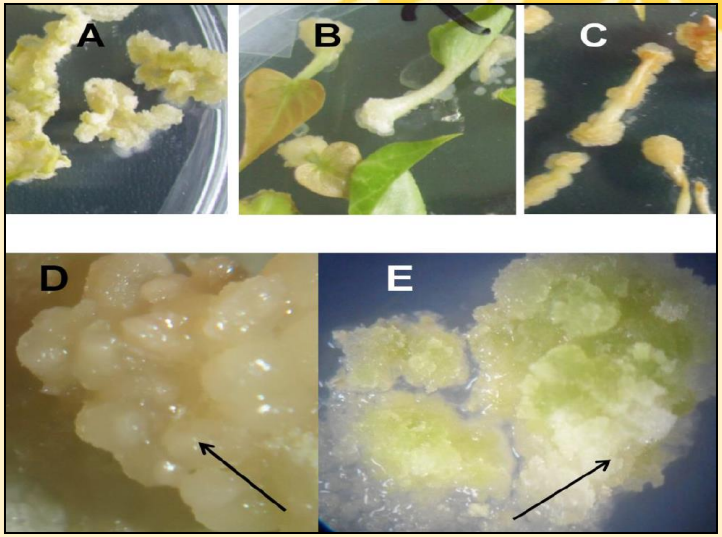


Wamalwa et al., 2013. Organogenesis and Agrobacterium-mediated Transformation of Several Sub-Saharan Africa Sweetpotato Cultivars. *Manuscript in prep*.

Genetic transformation update

At NaCRRI:

- Somatic embryogenesis works best with Kyebandula and Bwanjule
- Staff changes (PhD completion and return to Malawi by A. Sefasi)
- New technician and research assistant to continue with new *cry* gene constructs



✓ Sefasi et al., 2012. Induction of somatic embryogenesis in recalcitrant sweetpotato (Ipomoea batatas L.) cultivars. African Journal of Biotechnology, 11(94), 16055-16064

✓ Sefasi et al., 2013. Thidiazuron improves adventitious bud and shoot regeneration in recalcitrant sweetpotato. African Crop Science Journal 21: 85-95

Screening for weevil resistance

Four bioassay methods were evaluated on 31 transgenic events of Jewel carrying *cry* genes:

(A) artificial diet assay using transgenic root powder

(B) whole root assay

C) root chip assay

(D) root egg-plug assay

Results from weevil resistance

- Whole root infested with adult females and the egg-plug assays were the most appropriate of the 4 methods
- All 31 events were tested with various bioassays and resulted all with no mortality

TABLE 2. Mortality and development of *C. puncticollis* on artificial diet with lyophilised root powder of transgenic and non transgenic sweetpotato plants

Genotype	Mortality rate (% ±SE)	Pupation rate (% ±SE)	Larval weight (mg)
Transgenic			
CIP410008.7	26.7(±6.7)	23.61 (±6.05)	1.1
CIP410008.9	23.3(±3.3)	30.36(±3.72)	1.2
CIP410008.17	16.7(±3.3	31.94(±3.68)	1.0
CIP410009.5	30.0(±5.8)	28.97(±2.41)	1.2
CIP410009.12	16.7(±3.3)	36.11(±7.35)	1.2
CIP410010.14	26.7(±3.3)	27.38(±1.19)	1.3
CIP410010.18	30.0(±5.8)	24.21(±5.51)	1.0
CIP410010.19	30.0(±5.8)	29.56(±8.96)	1.2
Non-transgenic			
Cv Jewel	23.33(±3.3)	26.19(±7.31)	1.1

Rukarwa et al., 2013. Evaluation of bioassays for testing Bt sweetpotato events against sweetpotato weevils. African Crop Science Journal 21, no. 3: 235-244

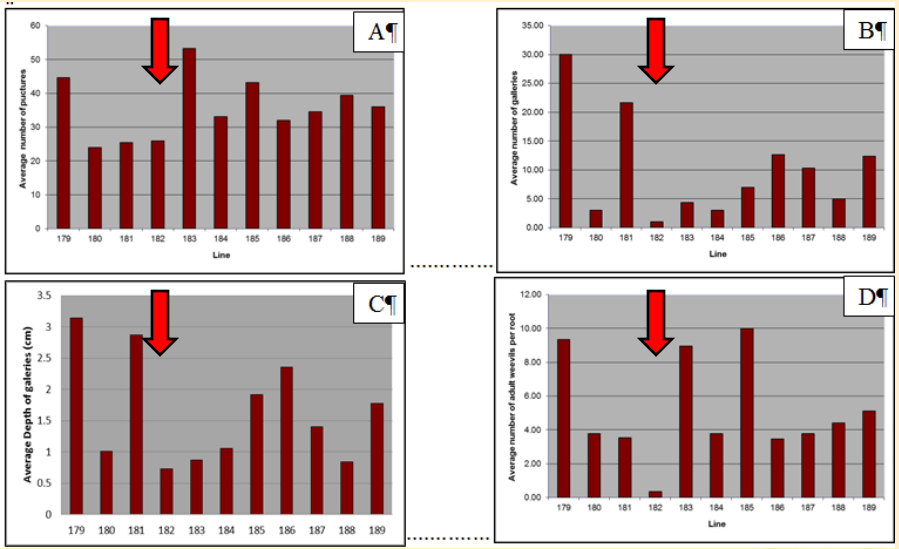
Confined field trial

- Puerto Rico:
  - Dimuth Siritunga and Fernando Gallardo at the University of Puerto Rico Mayaguez
  - 9 transgenic events with 2 WT lines planted on the 23rd of August, 2012 in the Isabel Agricultural research station.
  - Compliant with USDA-APHIS
  - 1000 lab-reared adult SP weevils released and infected roots

Sub-lethal activity of one TE?

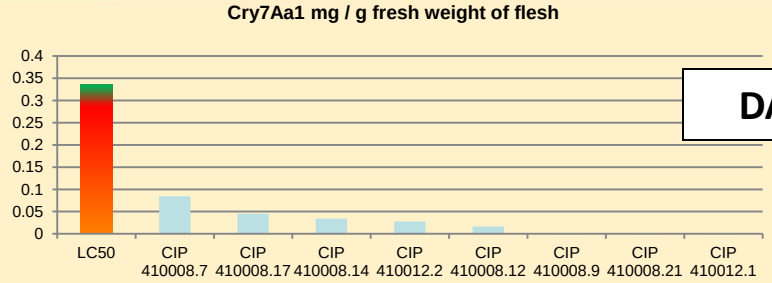
Entomologist at UPRM observed one TE (#182 with sub-lethal activity against *C. formicarius*:

- It carries the *cry3Ca1* gene
- It had consistently less infection than other TE and control
- Same sub-lethal activity of *cry3A* gene observed by Garcia et al., 2000
- BUT results do not have statistical significance

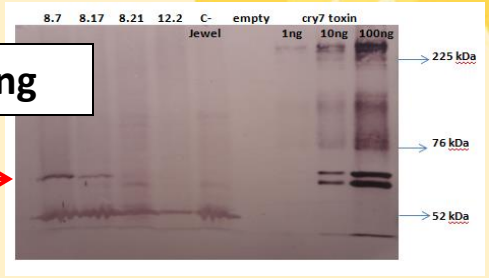


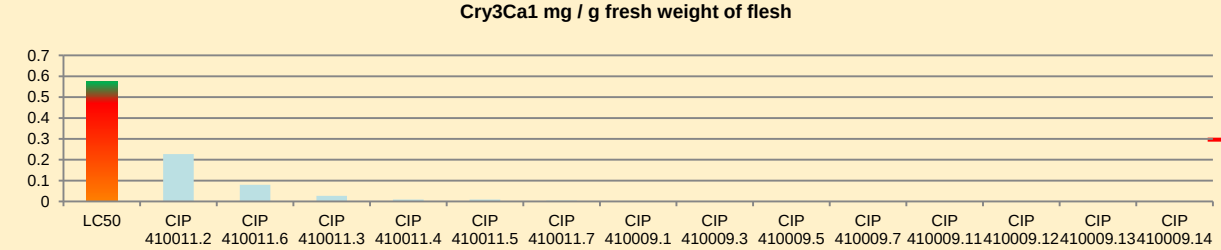
A. Average number of punctures per root; B. Average number of galleries; C. Depth of the galleries; D. Emerging weevils from the roots

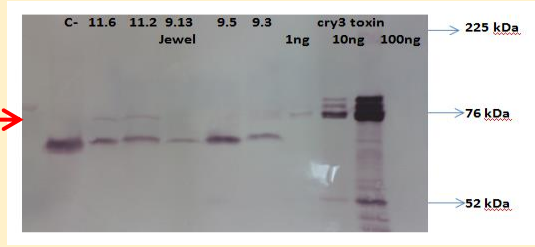
Cry protein accumulation

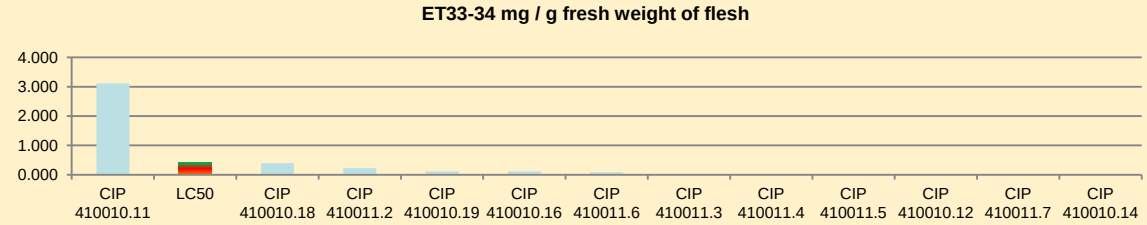


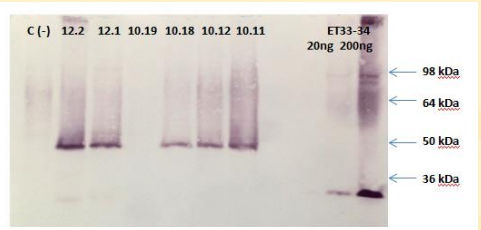
DAS-ELISA & Western blotting











- Cry7Aa1, Cry3Ca1, ET33-34, proteins are of the expected size (Western blot) and band intensity correlates with DAS-ELISA data. Low accumulation

High Cry protein expressers

- Many more events have to be screened especially those with higher levels of Cry protein accumulation.
- 33 events from an African cultivar Imby with *cry7Aa1* and *cry3Ca1* genes to be screened for weevil resistance after protein quantification
- 31 transgenic events [440163 (3), Jonathan (20), Huachano (8)] with same *cry* genes will be screened for resistance

Imby variety

New *cry* gene constructs

- The *cry* genes were constructed based on **sweetpotato-like gene** concept:
  - SP promoters, 5'UTR, 3'UTR, and poly-adenylation sequences from two SP genes β-amylase and sporamin, codon-optimised for SP, and the toxic fragment
- Because we observed low accumulation, new *cry* genes have been constructed based on maximising chances of high gene expression:
  - Double enhancer 35s and sporamin promoters, PVA or TEV translation enhancers, full protein of Cry7Aa1 (except the 501 aa C-terminal) and Cry3Ca1, and ET33 and ET34 separately
- 7 new *cry* gene constructs

#	Plasmid Name	backbone vector	Gene 1	Gene 2	Resistance gene	E.coli Strain	Agro Strain
1	pCIP104	pUC19	P35s : ePVA 5'UTR : Cry7Aa1 : Tβ-amyl		-	DH10β	-
2	pCIP105	pUC19	P35s : PVA 5'UTR : Cry3Ca1 : TSpoA1		-	DH10β	-
3	pCIP106	pUC19	Pβ-amy :PVA 5'UTR : Cry7Aa1 : Tβ-amy		-	DH10β	-
4	pCIP107	pUC19	PSpoA1 : PVA 5'UTR : Cry3Ca1 : TSpoA1		-	DH10β	-
5	pCIP108	pCAMBIA2305.1	P35s : PVA 5'UTR : Cry7Aa1 : Tβ-amyl		Km	DH10β	EHA105
6	pCIP109	pCAMBIA2305.1	P35s : PVA 5'UTR : Cry3Ca1 : TSpoA1		Km	DH10β	EHA106
7	pCIP110	pCAMBIA2305.1	Pβ-amyl : ePVA 5'UTR : Cry7Aa1 : Tβ-amyl		Km	DH10β	EHA107
8	pCIP111	pCAMBIA2305.1	PSpoA1 : PVA 5'UTR : Cry3Ca1 : TSpoA1		Km	DH10β	EHA108
9	pCIP112	pCAMBIA2305.1	P35s : PVA 5'UTR : Cry7Aa1 : Tβ-amyl	P35s : PVA 5'UTR : Cry3Ca1 : TSpoA1	Km	DH10β	EHA109
10	pCIP113	pCAMBIA2305.1	Pβ-amyl : ePVA 5'UTR : Cry7Aa1 : Tβ-amyl	PSpoA1 : PVA 5'UTR : Cry3Ca1 : TSpoA1	Km	DH10β	EHA110
11	pCIP114	pCAMBIA2305.1	PSpoA1 : PVA5'UTR : cryEt33 : TSpoA1	P35s : TEV : cryET34 : T35s	Km	DH10β	EHA111

Transient gene expression

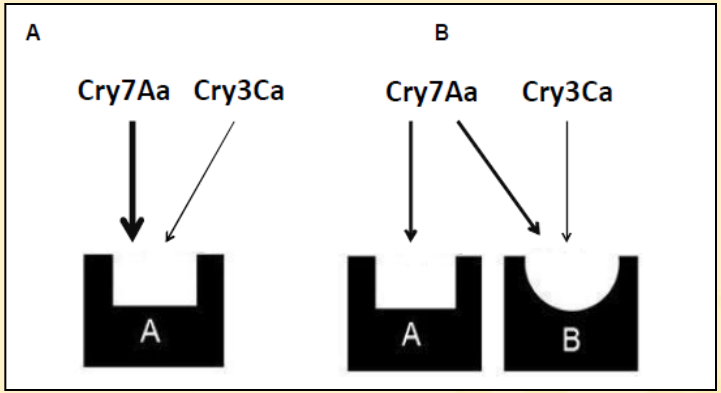
Testing the new (and old) gene constructs to quickly assessed which gene construct will produce the highest amount of Cry protein in storage roots:

- Agro-infiltration with vacuum could not detect Cry proteins with DAS-ELISA
- Biolistic to introduce plasmids into cells of storage root disks (University of Ghent and NARO-Kawanda)
- Agro-infiltration in *Nicotiana benthamiana* leaves (not suitable for root-specific promoter):
  - Both *cry7Aa1* and *cry3Ca1* genes are functional
  - *cry3Ca1* gene accumulates much more Cry protein

pCIP	2p35s:cry7Aa1	2p35s:cry3Ca1
	ug/gr	
112 S1 -Nb	0.162	2.918
112 S2 -Nb	0.094	0.426
112 S3 -Nb	0.117	3.434

Mode of action of Cry proteins

- At University of Valencia, results on proteinase activation of pro-toxins in three weevil species confirm that the *cry7Aa1* and *cry3Ca1* transgenes introduced in sweetpotato plants code for proteins fragments slightly longer than the ones with binding activity, suggesting that such fragments can become toxic for the insects after in vivo processing.
- The apparent competition for the same binding site of weevil midgut between the two toxins indicates that only one of them should be pursued for resistance management point of view.



Summary of results so far

- Have protocols for transformation of African varieties
- Have 64 TE under screening
- Have one TE with *cry3Ca1* possibly with sub-lethal activity
- Have 7 new gene construct
- Have Cry3Ca1 >>> Cry7Aa1 with d35s promoter
- Have competition between Cry3Ca1 and Cry7Aa1

Workplan till completion

- Confirm the sub lethal activity of the event with *cry3Ca1* gene in a confined field trial (UPRM-USA)
- Use biolistic for transient gene expression to assess level of expression between new and old gene constructs in storage roots (U Ghent-BE; NARO-UG)
- Characterize Bt strains for presence of *cry3Ca1*, *ET33 & ET34* genes and produce batches of the corresponding proteins (U Valencia-SP)

Workplan Cont'

- Transform with *cry3Ca1* and *ET33* and *ET34* genes one or two African varieties at ABL-Peru, BecA-Kenya, and NaCRRI-UG
- Identify high Cry protein accumulation in storage roots from at least 30 events per gene construct (3) (BecA-KE; ABL-PE)
- Evaluate efficacy of the transgenic events with the highest Cry protein content (*C. puncticollis* and *C. brunneus* (NARO-UG)

Workplan cont'

- Assess the efficacy of RNAi strategies against the weevils (see Prof Smagghe presentation, U Ghent-BE; NARO-UG)
- Establish a small scale bioassay for testing purified Cry proteins and dsRNA (U Ghent-BE; NARO-UG)

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Thank You