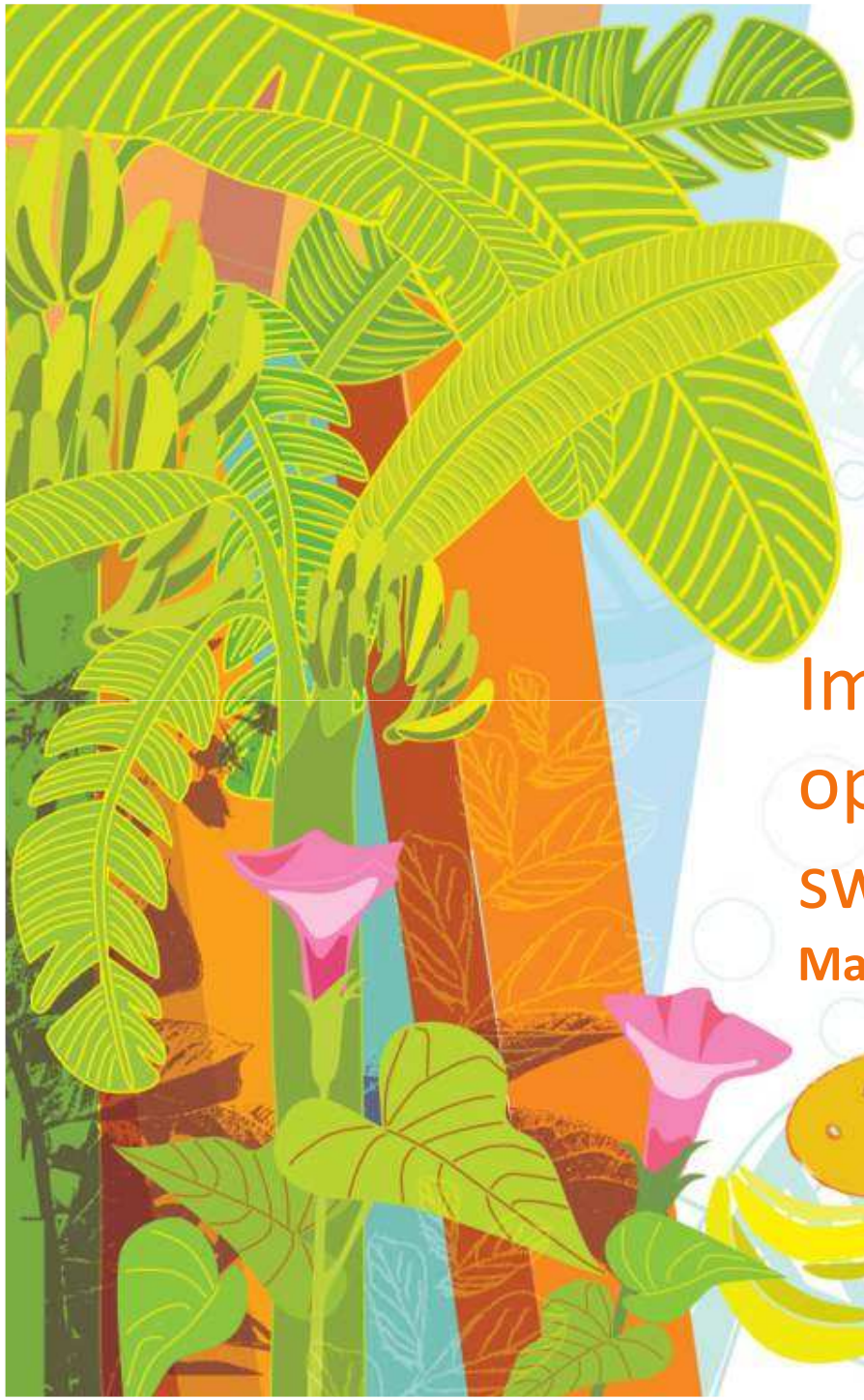


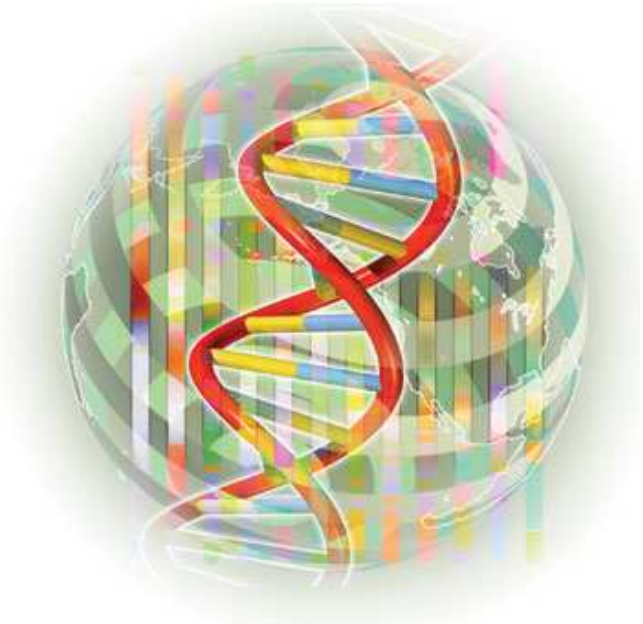
Improvement of Sweetpotato: opportunities for transgenic sweetpotato

Marc Ghislain

SP Breeding & Biotechnology
IPBO, Gents, August 20-31, 2011

Outline

- Gene-specific genetic modification:
  - Direct gene transfer – Transgenesis
  - Gene editing
- The economics of a GM sweetpotato
- The product development
- Priority traits:
  - weevils
  - virus disease
- Safety of GM sweetpotato
- Issues for discussion



Limitations of non-GM approaches

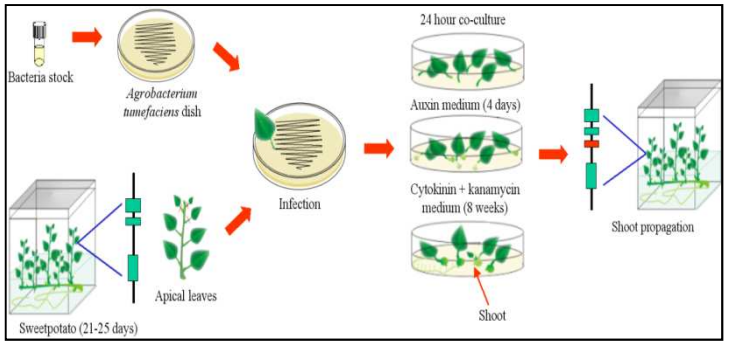
- Constraint within the own crop gene pool to find useful alleles (durable resistance in other species)
- Introduce genetic modifications that are not available for the gene of interest (mutations, silencing, ectopic and over-expression)
- Breeding systems of clonally-propagated crops can’t introgress useful alleles into existing varieties
- Many integrated crop management practices are knowledge intensive and site-specific
- Biological controls require products from local suppliers rarely available

Direct gene transfer - Transgenesis

- Biolistic / Gene Gun / Particle bombardment:
  - High velocity DNA-coated particles into cells, or embryos
  - Advantages:
    - no Agrobacterium bacteria infection and elimination;
    - Multiple inserts;
    - Organelle transformation;
    - Introduce unlinked genes in different loci
  - Disadvantages:
    - Requires breeding to eliminate non-functional insertions;
    - Patent restrictions

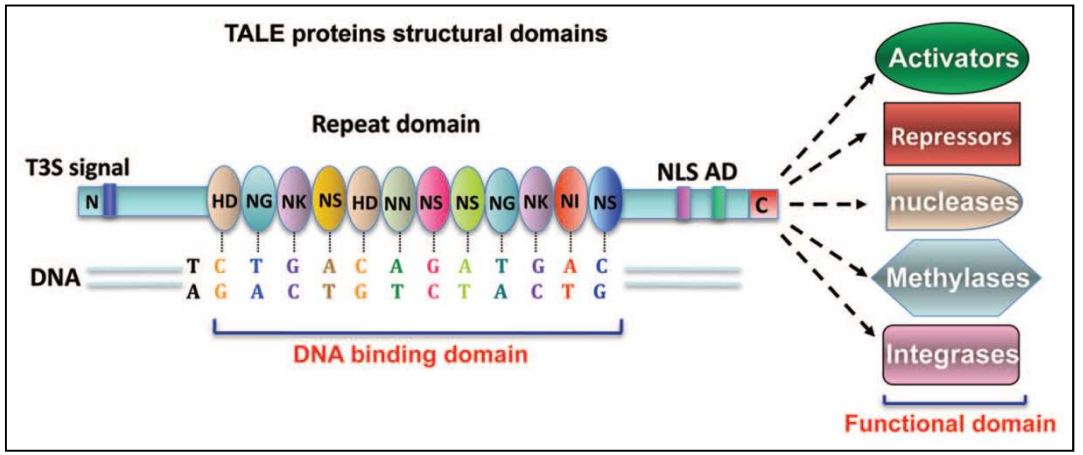
Direct gene transfer - Transgenesis

- Agrobacterium-mediated transfer:
  - Natural gene transfer between Agrobacterium tumefaciens and plant cell – modified to transfer only genes / DNA of interest
  - Advantages:
    - Relatively clean insertions;
    - One to five copies (tandems) / few loci;
    - 1 to 15 genes can be transferred
  - Disadvantages:
    - Elimination of Agro is sometimes cumbersome



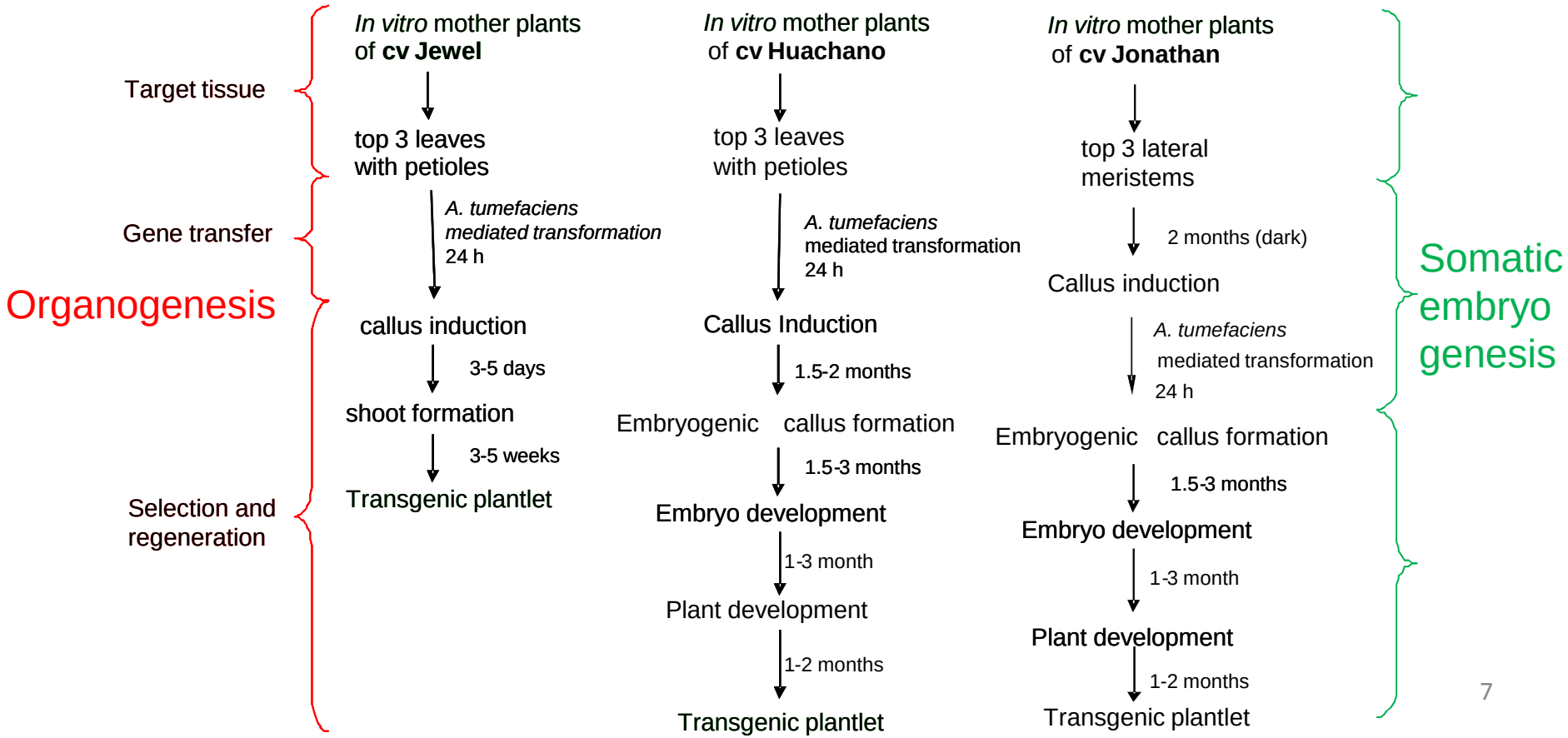
Gene/ome editing – TALE

- Transcription activator-like effectors (TALEs) proteins are used to provide highly specific and adaptable DNA binding modules which can then edit DNA (genes), knock off genes, add DNA (resistant alleles):
  - Advantages:
    - Targeted mutagenesis;
    - No regulatory burden (in theory)
  - Disadvantages:
    - Restricted to existing genes



Direct transfer into African varieties

- Two strategies for sweetpotato transformation:



Direct transfer into African varieties

- Organogenesis (ABL and BecA):
  - Out of 31 African varieties, 6 had regeneration efficiencies higher than 40% [Mugande, Imby, Luapula, Kawogo, Zambezi and Mafutha]
  - Ukerewe and Luapula have so far generated 2 putative transformed events using pCIP85.

- Conclusions:
  1. From this study, we would conclude that approximately 20% of African sweetpotato cultivars are amenable to transformation by organogenesis;
  2. However, the yield is still too low to be consider as the transformation method of choice for commercial product development.

Direct transfer into African varieties

- Somatic embryogenesis (ABL and Kawanda):
  - At ABL, the variety Imby from Burundi produced 31 independent events through s.e. after 8 to 12 months;
  - At Kawada, out of 11 Ugandan cvs established *in vitro* and regenerated through s. e. , 6 produced embryogenic callus, and 4 of them roots from callus
- Conclusions:
  - Large number of independent events can be produced but better African varieties need to be identified.
  - Test in liquid culture following Chinese protocols

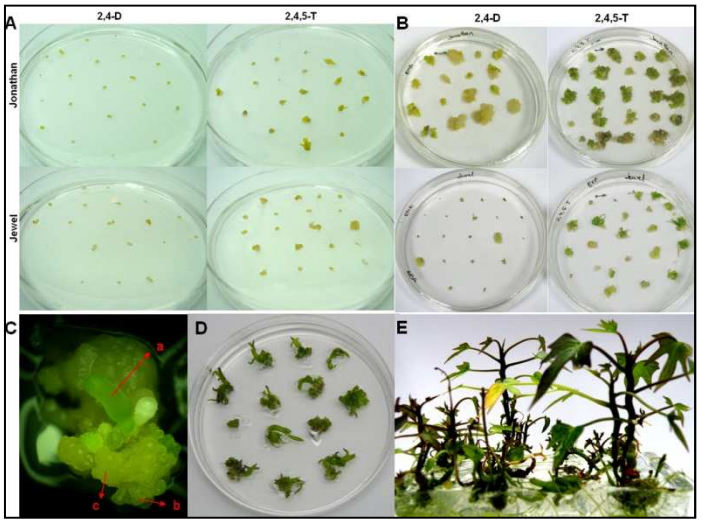
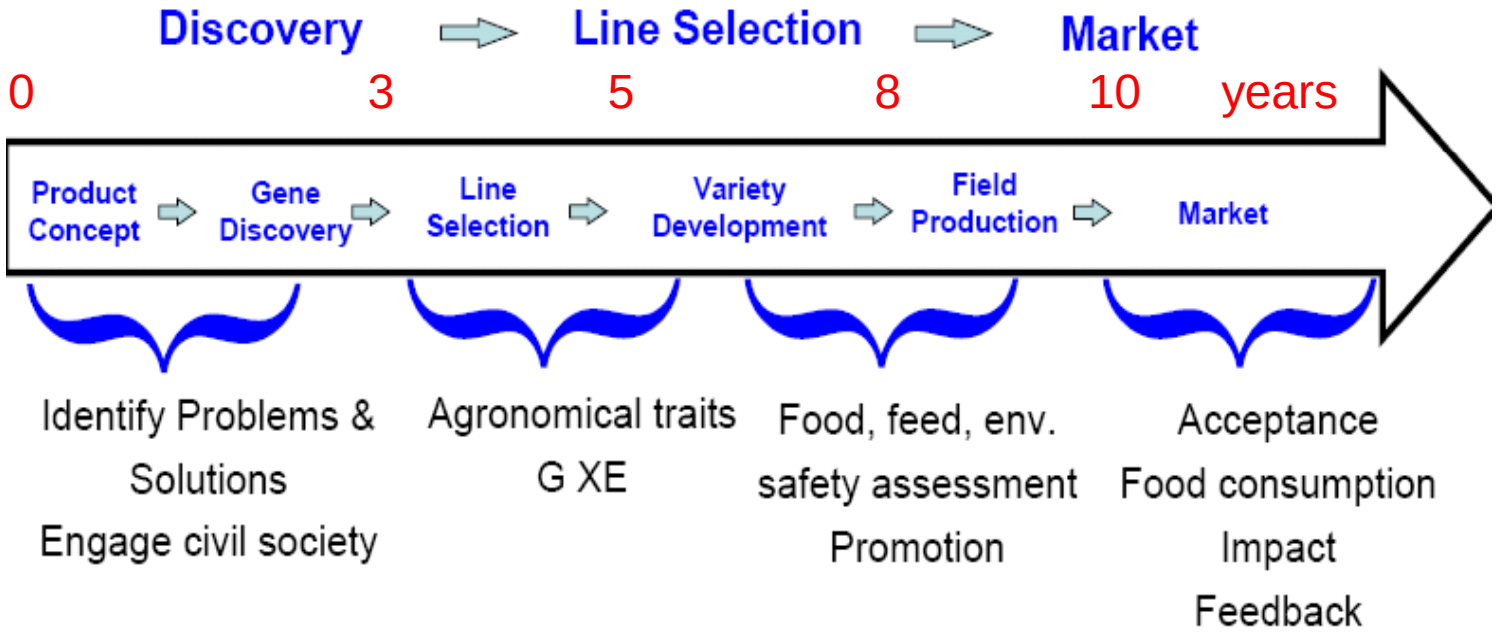


Figure 1. (A) (B) Calli formation after 4 and 8 weeks; (C) Embryogenic callus from meristem (a: green embryo at the torpedo stage, b: light green heart shaped embryo, c: yellow globular embryo); (D) (E) Regeneration from embryogenic calli.

Socio-economic considerations

- Kalaitzandonakes et al., 2007 estimated to 7-15 million USD the regulatory compliance cost of insect-resistant maize (private sector survey);
- Costs associated with product stewardship and post-release monitoring vary on the crop, trait, and country;
- Hence, GM approaches for non-profit organizations should be restricted to solving constraints with huge negative impact which can't be solved otherwise

GM product development



Priority traits:

1. Pest and disease resistance: weevils (Cylas spp.), virus disease (chlorotic stunt and feathery mottle viruses), nematodes ;
2. High yield under stress conditions: drought, heat, low input;
3. Improved nutritional quality: vitamins, minerals (iron, zinc), and proteins.

Weevil resistance in sweetpotato through biotechnology

Sweetpotato weevils, *Cylas puncticollis* and *C. brunneus* are among the top five priority constraints for sweetpotato production, rendering the crop unsuitable for human consumption. Particularly severe in drier zones.

Production losses due to weevil feeding may reach 60%-100%.

Background and justifications

- Survey on the socio-economic impact of weevils in Uganda (2007- 8) reports an average yield loss of over 28% between wet and dry seasons (Kiiza *et al*., 2009).
- IPM practices are difficult to implement in small-scale field with sweetpotato grown year round;
- Effective weevil resistance has not been found and used by conventional breeding;
- Bt crops have shown to be safe and have a positive impact on the environment while controlling targeted pests due to pesticide use reduction.

Kiiza *et al*., 2009. Uganda Country Report

Coleopteran pest resistant crops

- Colorado potato beetle resistant potatoes produced by inserting the ***cry3A*** gene from *Bacillus thuringiensis* (subsp. Tenebrionis) released in 1996 – 2004 by Monsanto.
- Corn rootworm-resistant maize produced by inserting the ***cry34Ab1*** and ***cry35Ab1*** genes from *Bacillus thuringiensis* strain PS149B1 released in 2005 by Dow.
- Corn rootworm resistant maize produced by transformation with a modified ***cry3A*** gene released in 2007 by Syngenta.
- Corn root worm resistant maize produced by inserting the ***cry3Bb1*** *gene from Bacillus thuringiensis subsp. Kumamotoensis* released in 2001 by Monsanto.

• Weevil resistant sweetpotato

Ex ante analysis of weevil and virus disease technology:

- Farmer survey 2007, 2008 in Burundi, DR Congo (Kivu), Uganda, Rwanda.
- 3 agro-ecologies, interview + field observations (regional analysis)
- Economic Surplus analysis
- Costs:
  - Research (2003-10) = US$ 3,092,567
  - Development (2011-15) = US$ 3,500,000
  - Deployment (2016-30) = US$ 9,750,000
- Gains (Uganda only):
  - 25% adoption: NPV = US$97,000,000 **IRR = 34%**
  - 10% adoption: NPV = US$36,000,000 **IRR = 27%**
- Discussion points: inclusion or not of research costs, better estimated costs, impact of spill-over, … ,
  IRR will only go up!

Damages caused by *Cylas* spp.2010

Cry proteins active against weevils

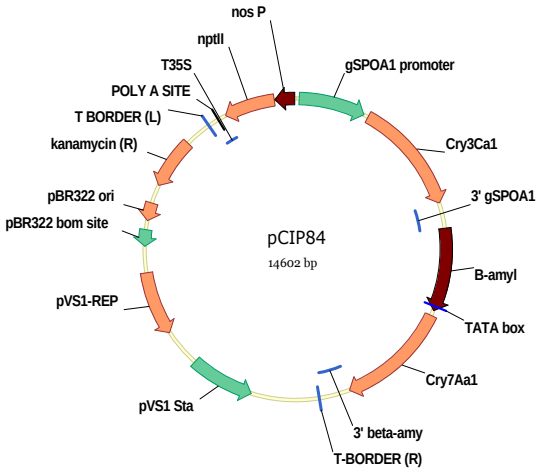
LC_{50} values (mg/ml diet) of Bt proteins against 1st instar *C. puncticollis* and 2nd instar *C. brunneus* (An LC_{50} below 1 ppm is low enough to expect high levels of toxicity when expressed in sweetpotato)

	Cylas puncticollis					*Cylas brunneus*				
Bt protein	Total n	LC_{50} Values	95% F.L	Slope (mean ± SE)	χ^2	Total n	LC_{50} Values	95% F.L	Slope (mean ± SE)	χ^2
Bt1 (ET33/34)	2760	0.417	0.394 - 0.441	2.733 ± 0.151	9.1	2760	0.458	0.411 - 0.502	2.636 ± 0.240	9.7
Bt2 (ET70)	2550	0.781	0.678 - 0.882	1.835 ± 0.095	24.0	2700	1.014	0.936 - 1.092	1.851 ± 0.091	11.3
Bt3 (Cry3Aa3)	2700	1.993	1.754 - 2.262	1.578 ± 0.131	14.2	2230	1.885	1.542 - 2.201	2.302 ± 0.204	17.0
Bt4 (Cry3Bb2)	2230	1.273	1.165 - 1.378	2.028 ± 0.101	13.9	2250	1.304	1.183 - 1.437	1.518 ± 0.116	6.4
Bt5 (Cry3Bb3)	2750	1.815	1.625 - 1.996	2.118 ± 0.138	21.0	2700	1.826	1.676 - 1.983	1.585 ± 0.097	10.2
Bt6 (Cry3Ca1)	2700	0.575	0.530 - 0.619	2.598 ± 0.112	20.6	2100	0.696	0.644 - 0.750	2.082 ± 0.119	6.5
Bt7 (Cry7A1)	2469	0.335	0.310 - 0.359	2.778 ± 0.178	8.0	2250	0.435	0.402 - 0.467	2.54 ± 0.178	3.8

Moses et al. (2010). Journal of Economic Entomology 103:1493-1502

Gene-protection technology

- Sweetpotato-like weevil resistance genes:
  - Promoter, untranslated, and the polyadenylation sequences from storage root expressed genes of sweetpotato (sporamin and β-amylase)
  - Codon usage of the sweetpotato
  - Protein identical to the protein produced by *B. thuringiensis*
- 5 WR or *cry* gene constructs:
  - *cry7Aa1*
  - *cry3Ca1*
  - *ET33-34*



Agrobacterium-mediated transformation:

- 34 events from Jewel, 5 from Huachano, 1 from Wagabolige, 40 from Imby, 10 from other SP varieties produced at ABL Peru
- 31 events multiplied at BecA and Kenyatta University
- Recently, 40 events from Imby

31 events from sweetpotato bearing WR genes at BecA – 2010

Agrobacterium mediated transformation:

- 39 sweetpotato events from Jewel were imported into Uganda and transplanted to the greenhouse at NaCRRI

Efficacy against weevils at NaCRRI

Artificial diet infested by 2nd instar larvae:

- delicate handling of larvae, high mortality, 8% DM, results with no statistical significance but no evidence of efficacy of none of the 29 events from Jewel.

Efficacy against African weevils – Assay 2-3-4

Whole root infested with female adults:

- need large number / no significant differences.

Root chips infested by plug with one egg:

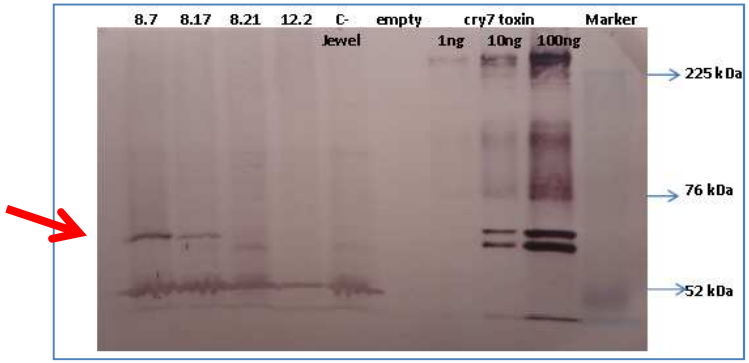
- difficult to control infection / difficult to determine mortality causes.

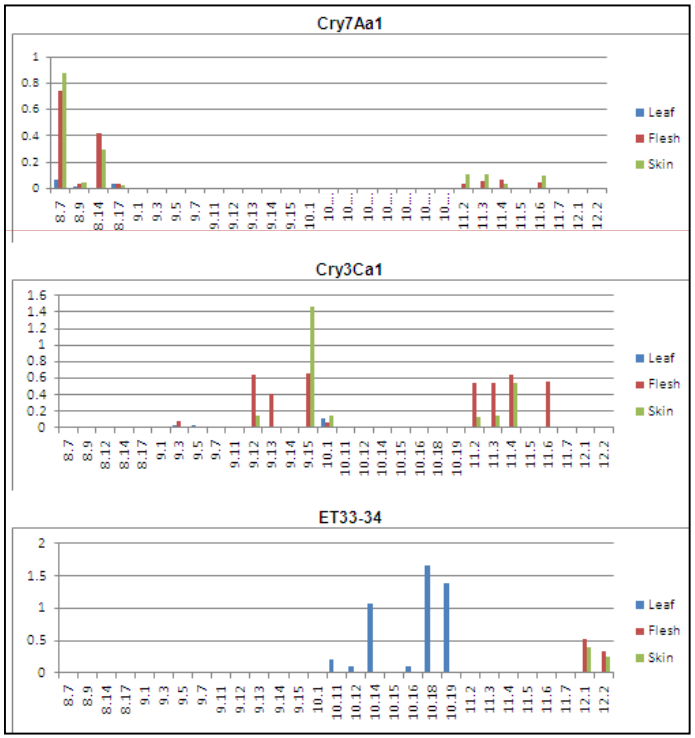
Small roots infested by plug with one egg:

- Plug of non transgenic flesh with two 24 hr eggs infested onto the transgenic root / Need additional repetitions.

Cry protein quantification

- Relative accumulation plot of Cry proteins in transgenic events from Jewel:
  - Quantification of Cry protein accumulation in leaves, skin and flesh of the storage roots;
  - No correlation between transcription in leaves and Cry protein accumulation in the storage roots;
  - Level of accumulation in the storage root flesh are for all but two inferior to LC50 (<1ppm)..





Next steps towards WRSP

- Where we are:
  - The *cry* genes transferred into SP are functional;
  - Few African SP varieties can be transformed;
  - Transgenic breeding is achievable
  - Capacity is now established at NaCRRI
- To do:
  - Screen more events for high expresser;
  - Confined field trial in Puerto Rico with 9 events from Jewel to look at mortality and sub-lethal activity.
  - New cry genes with high expresser features;
  - Test RNAi against weevils.

Transgenic Breeding

Rationale:

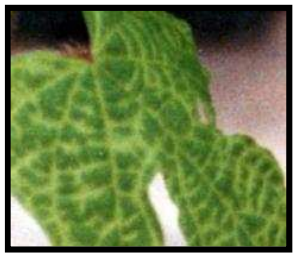
- We have obtained about 40 transgenic events from non-African varieties [Jewel, Huachano, Jonathan]
- After the identification of one event causing high mortality of weevils, we would cross it with African germplasm (=Transgenic breeding).

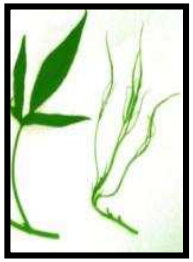
Crossing:

- Events with high accumulation of Cry protein grafted to *I. Setosa* to induce flowering;
- Crossing underway with New Kawogo, Tanzania and Naspot 1

Sweetpotato virus disease:

Economic Impact of SPVD:



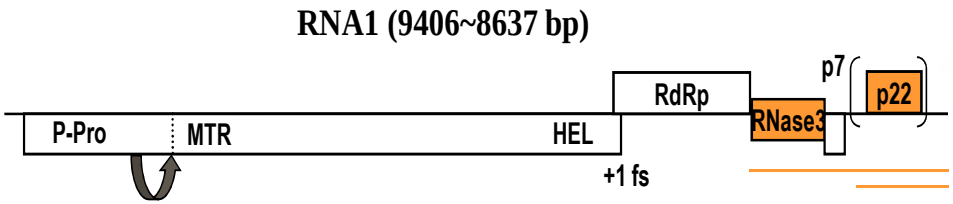



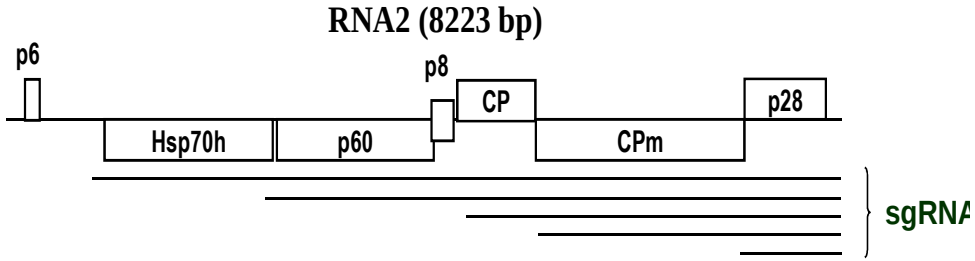

Symptoms	Yield Losses (Uganda)	Average incidence
Stunted plants	66% -Bitambi (Aldrich 1963)	Low (0-20%) N.E. Uganda
Leaves- small distorted	98% -Tanzania vs negative control	High (50-95%) Central, S. Uganda
narrow (strap-like)	(Gibson et al 1998)	Incidence & severity closely associated
Vein clearing	60-98% 36 families (360 genotypes)	with prevalence of white flies
Pale	(Mwanga et al. 2001)	(Aritua, Legg 1998; Gibson et al. 2000)
(Gibson et al. 1998)		

Goal of project DDPSC & CIP: test efficacy of two different transgenic approaches to provide resistance to natural infection in the field "- research trial NaCRRI"

SPCSV - (genus *Crinivirus*, *Closteroviridae*)

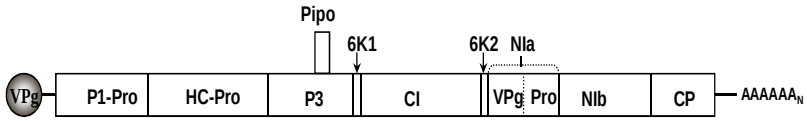
- Transmitted semi-persistently by whiteflies
- Phloem limited
- Two serotypes (EA, WA)





SPFMV - (genus *Potyvirus*, *Potyviridae*)

- Transmitted by aphids in non-persistent manner
- Single-stranded positive sense RNA genome of 10.8 - 11 Kb

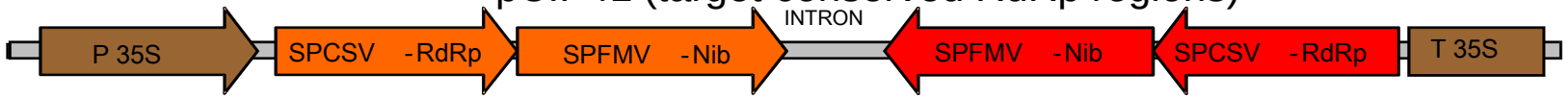


- Highly variable with four strain groups (C, RC, O & EA)

Target:
Virus resistance in sweetpotato

- Natural resistance to SPFMV and other viruses broken on co -infection with SPCSV
- Limited resistance to SPCSV known in sweetpotato germplasm
- Strategy for transgenic resistance: target both viruses using post post transcriptional gene silencing conferred by small interfering RNA (siRNA)
- Intron -spliced hairpin homologous to 3 alternative regions of both viruses:

pCIP41 (target conserved RdRp regions)

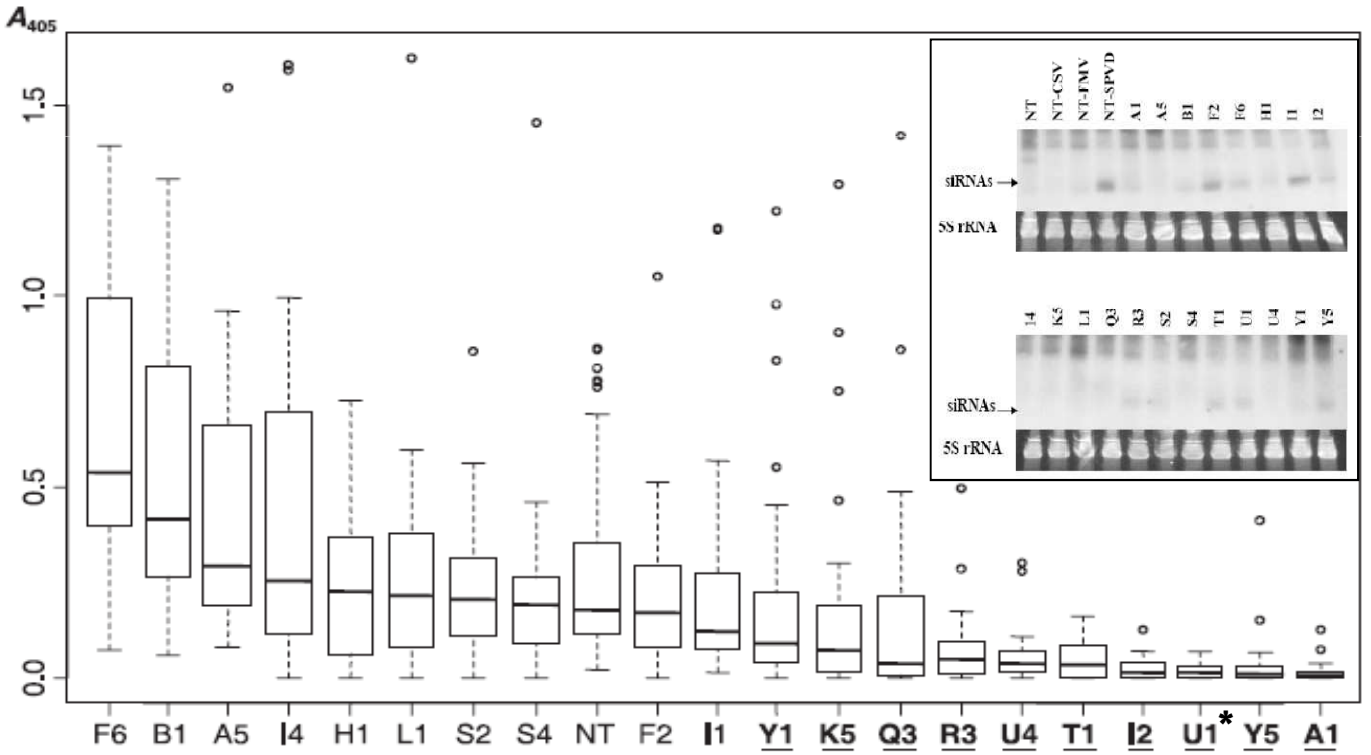


pC127 (regions with high levels of siRNAs produced under natural infections)

pC227 (regions with low levels of siRNAs produced under natural infections)

pCIP41

- 20 events: 50% produced virus specific siRNAs
- SPFMV: all plants resistant, including control
- SPCSV graft challenge: 50% significantly reduced virus titers

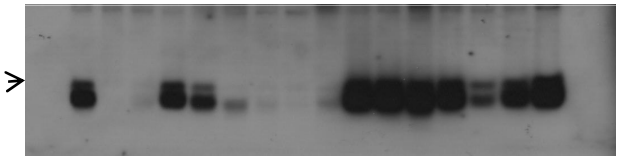


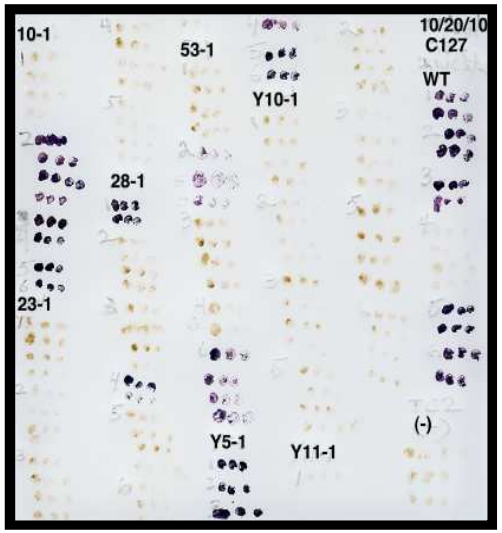
n=8
t=3

pC127 / 227

- virus specific siRNAs produced in most events
- Double infection with SPFMV and SPCSV resulted in some symptomless lines
- Dot blot hybridization for SPFMV these plants show undetectable virus titres

siRNA >





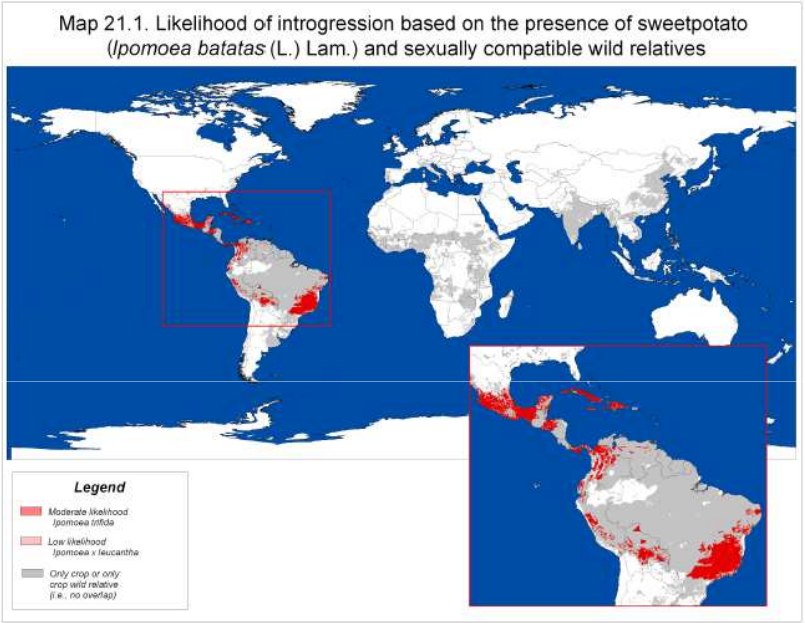
Safety to human

- History of safe use // Familiarity
- Protein allergenicity: assayed using two web search tools: SDAP - Structural Database of Allergenic Proteins and Allermatch. For both cases, FAO/WHO allergenicity assessment rules were applied, as well as procedures suggested by König *et al*. (2004) = 80 aa sliding window search and 8 aa epitopes
- Protein toxicity: assayed by amino acid sequence search with sequences deposited in in Genbank
- Stability, digestibility
- Mouse oral gavage study

Safety to the environment

- Environment:
  - No wild relatives in Africa
  - Gene flow var to var?
    - Infrequent successful out-crossing
    - Germination difficult
    - Fruit not attractive
    - Vine multiplication
    - Improved varieties

- Gene flow in sweetpotato is restricted to variety to variety which is not an issue for conventional varieties and has no negative environmental impact.



Andersson and de Vicente, 2008. Gene flow between crops and their wild relatives. Johns Hopkins University Press, Baltimore, Maryland, USA

Safety to the environment

- A review of non-target organism present in sweetpotato cropping system.
  - List of arthropods present in SP production system (literature, phytosanitary services, on-going GM deregulation, experts' opinion)
  - Coleopteran, other orders
  - Charismatic: honey bee, ladybird

- Only ladybird, ground, and rove beetles as coleopteran predators might need further investigation.

Issues for discussion: