The Sweetpotato Action for Security and Health in Africa (SASHA) is a five-year initiative designed to improve the food security and livelihoods of poor families in Sub-Saharan Africa by exploiting the untapped potential of sweetpotato. It will develop the essential capacities, products, and methods to reposition sweetpotato in food economies of Sub-Saharan African countries to alleviate poverty and undernutrition.

Development of Weevil Resistant Sweetpotato Varieties in Uganda using Biotechnology Tools

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

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

- 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

<table>
<thead>
<tr>
<th>Number of events</th>
<th>Gene</th>
<th>Binary plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td><em>cry7Aa1</em></td>
<td>pCIP78</td>
</tr>
<tr>
<td>9</td>
<td><em>cry3Ca1</em></td>
<td>pCIP79</td>
</tr>
<tr>
<td>8</td>
<td><em>ET33-34</em></td>
<td>pCIP82</td>
</tr>
<tr>
<td>6</td>
<td><em>cry7Aa1</em> + <em>cry3Ca1</em></td>
<td>pCIP84</td>
</tr>
<tr>
<td>2</td>
<td><em>cry7Aa1</em> + <em>ET33-34</em></td>
<td>pCIP85</td>
</tr>
</tbody>
</table>
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

![Graphs showing Cry7Aa1, Cry3Ca1, and ET33-34 protein levels in leaves, flesh, and skin over different events. Y axis = protein (as mg/g fresh weight). X axis = 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 2\textsuperscript{nd} instar larvae of \textit{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

<table>
<thead>
<tr>
<th>Family</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIP410008.7 x Naspot 11</td>
<td>12</td>
</tr>
<tr>
<td>CIP410008.7 x N/Kawogo</td>
<td>10</td>
</tr>
<tr>
<td>CIP410008.7 x Tanzania</td>
<td>16</td>
</tr>
<tr>
<td>CIP410010.19 x Naspot11</td>
<td>4</td>
</tr>
<tr>
<td>CIP410011.4  x N/Kawogo</td>
<td>4</td>
</tr>
<tr>
<td>CIP410012.2 x N/Kawogo</td>
<td>9</td>
</tr>
<tr>
<td>CIP410012.2 x Naspot 11</td>
<td>9</td>
</tr>
</tbody>
</table>
Way forward

- Selection of transgenic F1s: PCR analysis using specific primers for cry7Aa1, 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
Thank you