Suppression of Ubiquitin E2 Gene through RNA Interference causes Mortality in the Banana Weevil, *Cosmopolites sordidus* (Germar)

Introduction

- Banana weevil (BW) – the most important Musa pest in ECA.

- Larval stage most destructive.

- It has eluded control through cultural, chemical & biological options.

- No success yet reported through conventional & molecular options.
• We explored the potential use of RNAi as a biotechnology technique to suppress a key essential gene in the BW.

• It’s a natural mechanism triggered by dsRNA - regulating gene functions & protection of cells against foreign parasitic nucleic acids.

• Has been demonstrated in a range of organisms (including coleopterans) – with potential for pest and disease control.

• E.g. a bean variety resistant to golden mosaic virus developed through RNAi technique has been released.
Methodology

- Weevil DNA was extracted & purified.

- Identification of target genes and development of primers
  - *D. melanogaster* (D.m) essential gene Lwr (CsUBE) (DEG data base) used as a starting point.
  - blasted against *D. pendorosae* (D.p) & *T. casteneum* (T.c) genomes (in NCBI database)– identify Lwr orthologs
  - orthologs aligned & conserved gene portions surrounding portions with high RNAi activity designed into primers pairs.

- PCR products cleaned, cloned & sequenced
• Rightful clones confirmed with tblastx,(NCBI), transcribed into dsRNA

• C. sordidus 28S ribosomal RNA gene, partial sequence AY131082.1 used as a positive control

• dsRNA introduced into weevil larva through in-vitro feeding bioassays

Figure 1. A) 7 day old banana weevil eggs (red heads of larva visible); B) eggs in circular banana flower stalk tissues placed in a 24 well plate and C) a fully grown larva in a water control treatment.
• 20µl of dsRNA was applied at three concentrations - 10, 50 and 100ng/µl directly on the 5-6 day old eggs.

• 20µl nuclease free water served as a negative control

• Larva mortality & weight measured at 5, 8, 15 & 21 days.

• Growth rate measured between day 5 & 15

• ANOVA computed using GenStat, means separated with LSD at 5%

• Gene suppression levels determined using qRT-PCR at 6 and 10 days.
Results

• CsUBE dsRNA severely retarded growth (Fig. 2; Table 1)

• Larva were in most cases unable to bore into fresh tissue at 8 days

• Up to 100% mortality observed at 21 days at all concentrations of CsUBE (Table 2).

• LD50 was 1.8 ng/µl for this gene.

• Suppression levels of 6% and 11% observed at six & 10 days.
Table 1. Effect of dsRNAs on the growth rate (g/day) of banana weevil larva (measured between the 5th and 15th day). Significant differences (P <0.001) were observed between the genes.

<table>
<thead>
<tr>
<th>Gene ID/ Concentration</th>
<th>Growth rate (g x 10^-4/ day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 ng/µl</td>
</tr>
<tr>
<td>Control</td>
<td>17.86b,x</td>
</tr>
<tr>
<td>CsRIB</td>
<td>20.28b,xy</td>
</tr>
<tr>
<td>CsUBE</td>
<td>07.7a,x</td>
</tr>
</tbody>
</table>

Means followed by the same letters (a-b) in columns and (x-y) within rows are not significantly different at 5% LSD.
Table 2. Banana weevil larva mortality at 8, 15 and 21 days on banana flower stalk tissues treated with CsUBE dsRNA at 10, 50 and 100 ng/µl. Means are significantly different at P <0.001.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Mortality at 8 days</th>
<th>Mortality at 15 days</th>
<th>Mortality at 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (ng/µl)</td>
<td>Concentration (ng/µl)</td>
<td>Concentration (ng/µl)</td>
</tr>
<tr>
<td>Control</td>
<td>0a,x  0a,x  0a,x</td>
<td>0a,x  0a,x  0a,x</td>
<td>0a,x  0a,x  0a,x</td>
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<tr>
<td>CsRIB</td>
<td>0a,x  0a,x  0a,x</td>
<td>0a,x  0a,x  0a,x</td>
<td>0a,x  0a,x  0a,x</td>
</tr>
<tr>
<td>CsUBE</td>
<td>8a,x  10a,x  20a,x</td>
<td>49b,x  69b,y  70b,y</td>
<td>88b,x  100b,y  100b,y</td>
</tr>
</tbody>
</table>

Means with the same letters a-b in columns and x-y in rows are not significantly different at 5% LSD.
Discussions & conclusions

- CsUBE (UB E2) catabolizes proteins for growth & under stress conditions

- It involves the serial activation of three enzymes- ubiquitin-activating enzyme E1, E2 & the ubiquitin protein ligase family E3.

- E2 (CsUBE) mediates an irreversible reaction in this pathway

- E2 suppression– affected growth, development & other acute protein synthesis phases in the larva.
• For the first time, we demonstrate RNAi in the banana weevil & its potential to control the pest.

• *In planta* expression and evaluation of this dsRNA is recommended.
Thank you

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<table>
<thead>
<tr>
<th></th>
<th>Order</th>
<th>Family</th>
<th>Subfamily</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red flour beetle</td>
<td>Coleoptera</td>
<td>Tenebrionidae</td>
<td>Tenebrioninae</td>
<td>Tribolium</td>
<td>T. castaneum</td>
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<tr>
<td>Mountain pine beetle</td>
<td>Coleoptera</td>
<td>Curculionidae</td>
<td>Scolytinae</td>
<td>Dendroctonus</td>
<td>D. ponderosae</td>
</tr>
<tr>
<td>Banana weevil</td>
<td>Coleoptera</td>
<td>Curculionidae</td>
<td>Dryophthorinae</td>
<td>Cosmopolites</td>
<td>C. sordidus</td>
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</tbody>
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