Bananas: disease resistance through genetic modification

James Dale

Director, Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Australia
<table>
<thead>
<tr>
<th>Ranking</th>
<th>Crop</th>
<th>Production mt</th>
<th>Commercial production of GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn/maize</td>
<td>823</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Wheat</td>
<td>690</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Rice</td>
<td>685</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Potatoes</td>
<td>314</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Cassava</td>
<td>232</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Soybeans</td>
<td>230</td>
<td>+++</td>
</tr>
<tr>
<td>7</td>
<td>Banana/plantain</td>
<td>122</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Sweet potato</td>
<td>110</td>
<td>(+)</td>
</tr>
<tr>
<td>9</td>
<td>Sorghum</td>
<td>65</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Yams</td>
<td>51</td>
<td>-</td>
</tr>
</tbody>
</table>
The Origin of Bananas

Musa balbisiana (BB)

Musa acuminata (AA)
Most cultivated bananas are **triploids** derived from two wild progenitors: *Musa acuminata* and *M. balbisiana*

- Non-graminaceous monocots
- Large, herbaceous (non-woody)
  - pseudostem of leaf sheaths
- Cultivated bananas are parthenocarpic, low fertility
- Perennial growth
- Naturally propagated by suckers
- Fruit year round in tropics
Bananas

- In top 10 world crops
- World’s largest fruit crop in terms of both volume and value
- A dessert crop in the west
- A key dietary component in nearly all countries in the wet tropics
- ~100 million tonnes annual production
  - 1/3 each from Africa, Asia-Pacific, Americas
  - 85% consumed/traded locally
  - 15% for export trade
- Consumption highest in Africa’s great lakes region and Papua New Guinea
  - Up to 1kg/person/day
The problem for bananas

- The vast majority of domesticated bananas are selections from the wild, not bred: >300 different varieties and landraces

- Domesticated bananas are essentially sterile

As a result:

- Unlike nearly all other major crops, bananas have not been improved for thousands of years

- Because they are sterile they are exceptionally difficult to breed conventionally

- They have major deficiencies: diseases and nutritional value
### Annual Banana Production

**Million metric tonnes**

<table>
<thead>
<tr>
<th>Country</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>26.2</td>
</tr>
<tr>
<td>Uganda</td>
<td>10.5</td>
</tr>
<tr>
<td>Philippines</td>
<td>9.0</td>
</tr>
<tr>
<td>China</td>
<td>8.2</td>
</tr>
<tr>
<td>Ecuador</td>
<td>7.6</td>
</tr>
<tr>
<td>Brazil</td>
<td>7.2</td>
</tr>
<tr>
<td>Indonesia</td>
<td>6.3</td>
</tr>
<tr>
<td>Mexico</td>
<td>2.2</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>2.1</td>
</tr>
<tr>
<td>Colombia</td>
<td>2.0</td>
</tr>
<tr>
<td>Thailand</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Countries in bold non-export bananas**
Bananas (matooke) in Uganda: An integral part of the culture
The major banana diseases

- Black & Yellow Sigatoka
- Fusarium wilt race 1 & tropical race 4
- Banana bunchy top
- Bacterial wilt & Moko
- Nematodes
The major banana diseases

- Black & Yellow Sigatoka
- Fusarium wilt race 1 & tropical race 4
- Banana bunchy top
- Bacterial wilt & Moko
- Nematodes
Banana bunchy top virus
Banana bunchy top virus
BBTV worldwide distribution
Bunchy top disease movement

- Infected suckers
- Infected tissue culture plantlets
- Aphids

*Pentalonia nigronervosa*
Progression of Banana Bunchy Top Virus in Africa

- 1901 - Egypt
Progression of Banana Bunchy Top Virus in Africa

- 1901 - Egypt
- 1950s – D.R. Congo
Progression of Banana Bunchy Top Virus in Africa

- 1901 - Egypt
- 1950s – D.R. Congo
- 1960s to 1980s
  - Burundi
  - Central African Republic
  - Equatorial Guinea
  - Gabon
  - Rep. Congo
  - Rwanda
Progression of Banana Bunchy Top Virus in Africa

- 1901 - Egypt
- 1950s – D.R. Congo
- 1960s to 1980s
  - Burundi
  - Central African Republic
  - Equatorial Guinea
  - Gabon
  - Rep. Congo
  - Rwanda
- 1990s – Malawi & Zambia

© www.freeworldmaps.net
Progression of Banana Bunchy Top Virus in Africa

- 1901 - Egypt
- 1950s – D.R. Congo
- 1960s to 1980s
  - Burundi
  - Central African Republic
  - Equatorial Guinea
  - Gabon
  - Rep. Congo
  - Rwanda
- 1990s – Malawi & Zambia
- 2000s – Angola, Cameroon & Mozambique

© www.freeworldmaps.net
Progression of Banana Bunchy Top Virus in Africa

- 1901 - Egypt
- 1950s – D.R. Congo
- 1960s to 1980s
  - Burundi
  - Central African Republic
  - Equatorial Guinea
  - Gabon
  - Rep. Congo
  - Rwanda
- 1990s – Malawi & Zambia
- 2000s – Angola, Cameroon & Mozambique
- 2012 – Benin and Nigeria

© www.freeworldmaps.net
Banana bunchy top virus in Malawi
BBTV virions: 18nm isometric particles
Banana bunchy top virus (BBTV)

- ssDNA virus
- Isometric virions (18-20 nm)
- Multicomponent genome
- Each DNA encodes a single ORF except DNA-R
- DNA-M involved in RNA silencing
Banana bunchy top virus isolates subgroups

Sequence similarities between the two groups is approximately 90% for DNA-R and 85% for DNA-N
Bunchy top control options

- Avoidance: virus-free planting material, quarantine
- Minimisation: rogueing
- Grow slow infecting cultivars
- Conventional breeding: no resistance genes have been identified in bananas
- Genetic modification
Strategies for GM resistance for BBTV

- RNA interference (natural process)
  - The plant equivalent of human immune response
  - Plants recognise the invading virus as a pathogen and set up an ongoing defense (often too late)
  - As with human vaccination, plants can be “pre-immunised”
Identification of target BBTV sequences

- Human immune response targets proteins; plant RNAi targets RNA

- Three BBTV genes were selected:
  - Rep gene mRNA: critical function in replication
  - MP gene mRNA: function in cell-to-cell spread and silencing suppression
  - NSP gene mRNA: nuclear shuttle protein moving ssDNA from nucleus to cytoplasm
Hairpin(hp) RNA constructs

(a). Rep 5’ hairpin

(b). Rep 5’ MP hairpin
BBTV RNAi resistance

- **Transgenes**
  - RNAi transgenes targeting: movement protein gene or replication gene

- **Glasshouse challenge**
  - Multiple independent lines identified with apparent immunity (all contain MP sequence)

- **Field trials**
  - First field trial planned for late 2015 in Malawi
Panama Disease
or
Fusarium wilt
Fusarium wilt or Panama disease of bananas

- One of the most **devastating diseases** of agricultural crops in history

- Caused by *Fusarium oxysporum fsp cubense*

- Soil inhabiting fungus that lives more than **50 years** in infested soil; there are no chemical controls

- Infects through the roots and kills susceptible cultivars

- **Race 1** caused the destruction of Gros Michel in Central and South America last century which lead to Cavendish as the replacement

- **Tropical race 4**: kills Cavendish and many other bananas: a repeat of the demise of Gros Michel?
Distribution of Fusarium wilt Race 1
Fusarium in Lady finger
Wild-type Lady Finger challenged with Race 1
GM Lady finger with a stress tolerance gene
Transgenic resistant Lady finger: inoculated

Non-transgenic susceptible Lady finger: inoculated

Fusarium wilt Race 1

Non-inoculated Lady finger
Panama Disease
tropical race 4

A deadly disease that can kill more than 40% of the bananas in the world and it is on the move.
Banana diseases

Yes, we have no bananas

A big export industry is battling for survival on two fronts

When bananas started to be widely exported in the 1870s, they were an exotic fruit. By the 1950s the fruit seriously a hit but never mind — it was a wonder of millions of fans from the tropics. Then Panama disease struck. The soft fungus spread through Central and South America, killing banana plants in its path. By the 1980s Gros Michel (big Michel), the variety accounting for 90% of all exports, was close to extinction. The export industry approached collapse.

But in the nick of time growers identified a resistant commercial variety called Cavendish. Compared with Gros Michel, it was small and bland. Gros Michel could be fumigated to maintain stocks and Cavendish could be packed in coldroom and shipped in pressurised refrigeration containers. But there was no other alternative. Soon Cavendish replaced Gros Michel as the world’s top banana; the variety now accounts for 95% of all exports.

Bananas are now the world’s most valuable fruit. Exports rose from 11.3m tonnes in 2004 to 19.9m in 2012. American eat more bananas than apples and oranges together. But once were the export industry is fighting to survive—and this time, on two fronts.

First, black Sigatoka, a disease which blackens leaves and can halve yields, is gaining the ascendency over the fungus used to combat the disease. It is normally controlled by spraying, mainly weekly, which means growers costs considerably. Now growers in some places are having to increase dosage substantially, suggesting that spraying costs could become a new, unaffordable threat. Second, Fusarium Wilt tropical race 4 (TR4) is destroying plantations in several countries. The disease has spread through Central and South America, producing outbreaks of disease have been reported in some countries.

The disease is now known to be a banana disease that is both resistant to the two diseases. A Cavendish farm decontaminated by the fungus used to combat the disease, is no longer controlled by spraying. A disease that is now known to be a banana disease that is both resistant to the two diseases. A Cavendish farm decontaminated by the fungus used to combat the disease, is no longer controlled by spraying.

**The Economist**
March, 2014

Fusarium wilt tropical race 4 (TR4)
Fusarium wilt tropical race 4 (TR4) in Mindanao, the Philippines, February, 2014
Distribution of Fusarium wilt Race 1 and Tropical Race 4

TR4: Indonesia, Malaysia, China, the Philippines, Australia, Mozambique, Pakistan, Jordan, Lebanon
Tropical Race 4 in Australia
Tropical Race 4 in Australia

- Present in the Northern Territory for more than 20 years: 2015 only one commercial plantation remained

- March, 2015: TR4 recorded in the Tully Valley for the first time
QUT TR4 R&D Program

• Commenced 2001: hunt for resistance genes

• Identified a potential resistance gene in a resistant wild banana, *Musa acuminata malaccensis* (one of 25,000)

• Transferred the resistance gene into Cavendish by genetic modification

• Also Cavendish modified with Ced9, an anti-apoptosis gene from *C. elegans*

• Planted the first field trial near Darwin January, 2012
Resistant & Susceptible Lines in Darwin

2 GM lines with no disease after 3 years
QUT’s banana R&D program

- Panama Disease TR4 resistant bananas for Asia, Africa and Australia
- Bunchy top (virus) resistant bananas for Asia, Africa and Australia
- Biofortified banana for Uganda and East Africa
Bananas : sustainability : GM

Panama Disease

Bunchy top disease

Cavendish  Gros Michel

And many others

Lakatan  Sukali Ndizi
Bananas: sustainability: GM

Panama Disease
Bunchy top disease

Cavendish  Gros Michel
Lakatan  Sukali Ndizi
And many others
Bananas: sustainability: GM

Panama Disease

Bunchy top disease

Cavendish, Gros Michel, Lakatan, Sukali Ndizi, and many others

Gros Michel®
Cavendish®
Lakatan®
Sukali Ndizi®
Local traits for local crops

- Bananas: no interest to big agbiotech
- Plant genes or plant virus sequences
- Bringing back or conserving genetic diversity
Our Researchers and Supporters

**QUT**
- James Dale
- Rob Harding
- Ben Dugdale
- J-Y Paul
- Anthony James
- Bulu Mlalazi
- Jen Kleidon
- Phuong Huang
- Benard Mwari
- Maiko Kato

**NARO (Biofortification)**
- Wilberforce Tushemereirwe
- Stephen Buah
- Priver Namanya
- Betty Magambo
- Ruth Mbabazi
- Moses Matovo

**Virus Resistance**
- Leena Tripathi (IITA Nairobi)
- Misheck Soko (DARS Malawi)

**Field Trails –**
- Jeff Daniels
- Mark Smith

**Financial Support**
- Bill and Melinda Gates Foundation
- UK Department for International Development
- Australian Research Council
- Queensland University of Technology
- Horticulture Australia Limited
- LaManna Bananas