GMOs in Horticulture – Exciting Opportunities or Dead End?  
A Case Study on Banana  

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GMO bananas?
There is no “GMO” banana on the market yet
Bananas
Importance of banana/plantains/cooking bananas

- Staple food for 400-1,000 million people
- Produced in >120 countries
- Banana and plantains (*Musa* spp.): Largest fruit crop in the world with annual production of 139 mtonnes (2012, FAO)(apple: 71 mtonnes)
- International banana trade: yearly turnover of ~6 billion USD
- Useful products: fruit, leaves, terminal buds, fibres, corm
Use of bananas: Starch corm (*Ensete*)
Use of bananas; fibres (*Musa textilis*)
Use of bananas; beer bananas (East-African Highland bananas)
Use of bananas; male flower = vegetable
Use of bananas; feed
Use of bananas; flowers
Use of bananas; fruit: desert banana, snack, cooking banana (some plantains), matoke banana
cultivars

M. acuminata
AA
- Parthenocarpy
  - Cultivars AA
    - Abnormal meiosis
      - AAA
        - RED LUGUGIRA
          - IBOTA
            - GROS MICHEL
              - OROTAVA
      - AAB
        - SILK POME PLANTAIN LAKNAO POPOULOU MYSORE
  - ABB
    - BLUGGOE AWAK

M. balbisiana
BB

- Cavendish - local consumption 28%
- Plantain (AAB) 21%
- Highland bananas (AAA) and other ABB cooking bananas 24%
- Gros Michel and other dessert bananas (AAA/AAB) 14%
- Cavendish - export 13%
FAOSTAT major production area

Cooking bananas

Sweet Bananas

M = Million, k = Thousand
Why is improvement of bananas necessary?
Problems: diseases

- **Fungi**
  - Sigatoka (*Mycosphaerella*)
  - Panama (*Fusarium*)

- **Viral**
  - Banana Bunchy Top Virus (BBTV)
  - Banana Streak Virus (BSV)
  - Banana Bract Mosaic Virus (BBMV)
  - Cucumber Mosaic Virus (CMV)

- **Bacteria**
  - Moko (*Pseudomonas*)
  - Xanthomonas

- **Nematodes**
  - *Radopholus similis*, *Pratylenchus goodeyi*
  - *P. coffeae*, *Helicotylus multicinctus*

- **Insects**
  - Banana weevil (*Cosmopolites sordidus*)
Other putative improvement points

• Higher production
• Better storage (shelf life)
• Fruit quality (fortification: Vitamines, Iron)
• Stress resistance
  – wind
  – salt
  – cold
  – Drought
• Banana as a biofactory (Edible vaccines)
Where do “new” cvs have to come from?

• ‘Forgotten’ cultivars
  – in situ (S-E Azië)
  – ex situ (gene banks)

• Classical improvement (using bananas with superior characteristics)
  – High degree of sterility
  – Huge surface needed

• “Modern improvement techniques” (using embryogenic cell suspensions)
  – Mutation breeding
  – Somatic hybridization
  – Genetic modification
GMO work in banana
Situation of banana GMO research?

Topic=(Plant) AND Topic=(genetic transformation OR genetic engineering or transgenic)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Nº of publications</th>
</tr>
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<tbody>
<tr>
<td>Banana</td>
<td>205 (84 technical papers)</td>
</tr>
<tr>
<td>Rice</td>
<td>5953 (30 X banana)</td>
</tr>
<tr>
<td>Tomato</td>
<td>3657</td>
</tr>
<tr>
<td>Apple</td>
<td>627</td>
</tr>
<tr>
<td>Papaya</td>
<td>265</td>
</tr>
<tr>
<td>Cassava</td>
<td>234</td>
</tr>
</tbody>
</table>

Banana biotech papers: India, USA, Belgium, Australia

**Banana “under-under-researched”**
<table>
<thead>
<tr>
<th>Fruit</th>
<th>Year 1995-2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana</td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td></td>
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<tr>
<td>Tomato</td>
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Embryogenic cell suspensions!!!!!
History

1993-1994: The first banana transformation trials (Panis et al., 1993; Sági et al., 1994).

1995: Two back-to-back publications were published in Bio/technology
  • Embryogenic cell suspension of the ABB cv ‘Bluggoe’ (Sagi et al., 1995)
  • Shoot meristematic tissues of the AAA cv ‘Grand Naine’ (May et al., 1995)

Since 2000: Introduction of genes with added value (published research)
  • Black and yellow leaf streak diseases (Kovács et al., 2013, Ghag et al., 2014, Mohandas et al., 2013)
  • Edible vaccines (Kumar et al., 2005)
  • Nematodes (Atkinson et al., 2004; Roderick et al., 2012)
  • Bacteria (Tripathi et al., 2010, Vishnevetsky et al., 2011)
  • Virusses (Shekhawat et al., 2012)
  • Panama disease (Hu et al., 2013, Paul et al., 2011, Chakrabarti et al., 2003)
  • Biofortification (Kumar et al., 2005, Dale et al. this meeting)
  • Drought (Xu et al., 2014)

Most of the transgenics never made it to the field!
Confined field trials
<table>
<thead>
<tr>
<th>Research</th>
<th>Resistance to fungi, nematodes and bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field trials</td>
<td>USA 4</td>
</tr>
<tr>
<td></td>
<td>Israel, Australia, Uganda</td>
</tr>
<tr>
<td>Approvals</td>
<td>None</td>
</tr>
<tr>
<td>Perspectives</td>
<td>A commercial use of GM bananas is possible on the mid-term.</td>
</tr>
</tbody>
</table>

Breeding Aims
- Overview
- Herbicide Resistance
- Pest Resistance
- Disease Resistance
- Plants with Altered Composition

GM Food and Feed: Labelling Guide
- These products must be labelled
- These products do not require labelling
- Labelling: Flavours,
Confined field trials with GMO bananas

• **Israel**
  • 2006
  • Rahan meristems / Dr. Eli Kayat
  • RNAi technology against pathogenic nematodes

• **Uganda**
  • 2009
  • NARO/KU.Leuven
  • Rice Chitinase/ Mycophaecella f.
  • Gros Michel (19 lines)
Mf leaf disc bioassay
Confined field trials with GMO bananas

- Uganda (Tripathi et al., 2010, 2012)
  - 2010
  - NARO/IITA
  - Hrap (Hypersensitive response-assisting protein) gene from sweet pepper against *Xanthomonas* wilt (BXW)
  - Result: 12 transgenic lines at the 2nd ratoon show absolute resistance
  - Further testing
  - Release 2017?
Confined field trials with GMO bananas

- **USA (Florida) / Israel (Vishnevetsky et al., 2011)**
  - 2011
  - Cavendish
  - Stacking of Endochitinase gene ThEn-42, Stilbene synthase (StSy) gene and Superoxide dismutase gene Cu,Zn-SOD
  - Results of 4 years field trial: Resistance to Mycosphaerella fijiensis and Resistance to Botrytis cinerea

- **Australia**
  - 2011
  - QUT/ Dr. James Dale
  - Fusarium (Trop race 4) resistance
  - Dwarf cavendish, Williams, Grande Naine
Confined field trials with GMO Bananas

Australia/Uganda (Dale et al., 2013; Mlalazi et al., 2012)

- 2011
- QUT/ NARO/ Dr. James Dale/South Johnston
- enhanced nutrition (Vit A and iron)
- 1241 GM banana lines (Dwarf cavendish, Williams, Lady Finger)
- Originally genes from other plants but now banana gene, PSY (phytoene synthase), (from Orange pulp banana Auspina)
- Funded by BMGF
- Result 15X higher provitamine A level
- Aim: to grow the bananas in Uganda by 2020 after human trails in US (Ohio).
Confined field trials with banana (not many details available)

Uganda (Namuddu et al., 2013)

- 2012
- NARO
- Nematode resistance
- Sukali Ndiizi (ABB) with a modified Carica papaya cystatin (CpCYS) gene
Conclusions future of banana GMOs: Exciting Opportunities or Dead End?
Why bother?

(i) Bananas are susceptible to a wide range of pests and diseases

(ii) They constitute the Nº 1 fresh fruit crop in the world

(iii) They are highly sterile, making classical breeding extremely difficult but at the same time preventing transgene drift via pollen into the environment

(iv) Their cultivation is monoclonal
Difficulties

(i) Most efficient transformation protocols require embryogenic cell suspensions

(ii) Lack of standardized disease bioassays and correlation with field resistance

(iii) Until 2012 banana was not sequenced. In 2012 and 2013 the A and B genomes became available.

(iv) Time needed to obtain fully transformed plant that can be transferred to the field (at least 8 months)
Difficulties

(v) Cost and effort of maintaining transgenic lines (only in vitro: no cheaper (seed) storage possible)

(vi) Lack of a solid legal biosafety environment for culturing GMOs in many banana producing countries

(vii) Size of individual banana plants rendering large scale testing of transgenes extremely costly

(viii) Lack of funds
   (i) Classical banana industry rather conservative
   (ii) Mostly grown in “poor” countries
Exciting Opportunities or Dead End?

It is just starting!

- Be open about the results of field trials. Also report negative results. It helps build up trust.
- Be realistic: do not say “GMO bananas will save the world” or “without GMOs bananas will disappear”.
- Explain what GMOs are. Also be open about “eventual” risks.
- Make a good product that growers/consumers want. Too easy to say that because of the anti GMO lobby there is no GMO banana on the market. Is there something marketable?