Viral infection and nicotine conversion to nornicotine in the next generation.

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Nicotine conversion to nornicotine

- May occur during leaf yellowing & curing.
- Converter plants may be found in the descent of non converter plants
  - More frequent in burley.
  - The nicotine demethylase gene is present in any plant, converter or not.
  - In non converters, its expression is blocked
    - Epigenetic event(s) would allow the expression of the nicotine demethylase gene in part of the next generation?
    - What is the cause of such events?
Converter tobacco

Leaf maturity

Expression
Translation

CYP 82 E4

Nicotine demethylase enzyme

Nicotine
tyellowing

Nornicotine
Non converter tobacco

Leaf maturity

CYP 82 E4

yellowing

Nicotine demethylase enzyme

Nicotine

Nornicotine
What would be such epigenetic change and how would it be triggered?

- **DNA recombination?**
  - The progeny of TMV-infected plants exhibited an over 3 fold increase of inherited changes in the luciferase transgene
    - Kolwalchuk I. et al., 2003

- **DNA methylation?**
  - TYLC Sardinia virus infection on tomato led to DNA methylation of genes involved in virus defense response.
    - Mason G. et al., 2008.

- Virus infection? Other stresses? ?
The experiment

- 2006: producing progenies from the burley TN 90LC virus infected / non-infected plants
- 2008 and 2010
  - Field tests in an attempt to estimating the frequencies of converter plants in each kind of progeny
• Bergerac 2006
• Inoculated in the field 2 weeks after transplantation
• $\text{PVY}^N$, CMV DTL
• Symptoms + ELISA tests confirmed inoculation success
Self pollinated 10 shoots / treatment

- Harvested and cleaned separately the seeds
- from each individual shoot
- in each treatment
Bulks

- PVY seed bulk
  - Equal amount of seeds from each of the 10 PVYN inoculated shoots
    - Bulked → next generation « TN90 PVY »

- Same for CMV, CMV + PVY, mock and non inoculated
Studying bulked progenies

- Tranplantation: May, grown as usual, untopped
- At maturity (September), harvested 2 middle leaves on each shoot
  - Air cured
  - Yellowing as complete as possible
  - Ground to powder (whole leaf)
  - Alkaloid extraction
  - HPLC analysis
Estimating nicotine conversion
HPLC, converter plant

Internal standard
Quinine

nicotine

nornicotine
## Number of plants studied / bulk

<table>
<thead>
<tr>
<th></th>
<th>Non inoculated</th>
<th>Mock</th>
<th>CMV</th>
<th>PVY</th>
<th>CMV + PVY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>2010</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
2008 results

Conversion ratio

CMV+PVY

Non inoc
2008 result (2)

Conversion ratio

MOCK

Non inoc
### 2008 results – stat.

<table>
<thead>
<tr>
<th></th>
<th>total shoots analysed</th>
<th>Frequency of converters (ratio &gt; 10%)</th>
<th>Frequency of converters (ratio &gt; 15%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non inoculated</td>
<td>94</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Mock</td>
<td>99</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>CMV+PVY</td>
<td>193</td>
<td>0.26</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Red = significant difference from non inoculated (5%)
2010 results -1

![Conversion ratio graphs for pvy and mock]
2010 results - 2

Conversion ratio
0
10
20
30
40
50
60
70
80
90
100

cmv

Conversion ratio
0
10
20
30
40
50
60
70
80
90
100

mock
2010 results – stat.

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<td>PVY</td>
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<td>0.26</td>
<td>0.19</td>
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Red= significant difference from Mock (5%)
Conclusion

- Viral infections significantly increased the frequency of converter plants in progenies.
  - CMV+PVY: 2 – 3 fold
  - PVY: 2 fold
  - CMV: 1.5 fold (ns).
- Discard virus infected plants in seed production
  - As soon as possible
  - Foundation seeds and seed production
Perspectives

- Use of epigenetic induced changes in breeding?
  - May well be already the case in a non intended, non conscious way.
  - If conscious, could be optimised
    - Suggested in Mason et al. 2008: molecular tools to identify methylated genes
    - Review: Boyko & Kovalchuk 2010

- Maintenance of true-to-type inbred lines: avoid epigenetic changes