Genetic engineering is a technology derived from the ideology of the “central dogma” of Molecular Biology according to which histories of organisms are pre-determined by genes seen as independent entities just as the components of a machine. On this ground genetic engineers thought that single genes from one genome could be transferred from one specie to another where they would perform the same function without any unintended effect.
Living systems are all organised as networks of molecules, cells, tissues, organisms, ecosystems, the Biosphere. All elements at all levels of the hierarchical organisation are interconnected. For this reason the change of one component always implies changes in the other nodes connected with it with partially unpredictable results. Genetic engineering therefore has a high level of unpredictability.
A gene and metabolic network with a modular structure as all living networks
Inserting a gene from the genome of one organism to that of another often far from the first in phylogenetic terms has several unintended consequences due to the fact that it did not co-evolve with the receiving genotype. If a relevant node is changed there will be pleiotropic effects while, depending on the sequences in which the transgene is transferred, position effects can change regulation.
The so-called “unintended effects” of genetic engineering are all derived by the dynamic interactions between the inserted construct and the context at different levels of organisation.

-- loosening of developmental constraints during in vitro culture leading to mutations

-- quasi-random location of inserted genes leading to “position effects”

-- production of “fusion RNAs” and putative new proteins

-- “active” re-arrangements and regulation of expression by host organisms

-- Un-predictable interactions with host metabolic networks leading to quantitative and qualitative changes in the transcriptome, proteome and metabolome

-- Interaction of the GMO with the agro-ecosystem

-- Effects on health of humans and animals

EFSA guidelines are not sufficient for a correct monitoring of unintended effects in these fields
In a transformation experiment it is impossible to predict where in the genome will be inserted disrupting the pre-existing sequence. EFSA guidelines suggest obsolete techniques for this analysis.
It is impossible to predict whether and how the transgene construct will be modified. Here we show:
The original construct (a) and nine different re-arranged sequences found in a single transformed oat line EFSA guidelines do not necessarily imply the analysis for the presence of unintended fragments.
If one looks at authorised GMPs present in the market one finds a number of unintended changes in the genome. We shall take two insect resistant Maize events and particularly MON 810 to show them.

<table>
<thead>
<tr>
<th>Transformed line or transformation event</th>
<th>Transformation method*</th>
<th>USDA application number and other relevant references</th>
<th>Date of approval for deregulation</th>
<th>Superfluous DNA at or near the transgene locus</th>
<th>Sequence analysis of DNA flanking the inserted transgene</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLCotton25</td>
<td>Agrobacterium-mediated transformation</td>
<td>02-042-01p</td>
<td>March 2003</td>
<td>Polylinker sequence</td>
<td>n.d.</td>
</tr>
<tr>
<td>Maize MON883</td>
<td>Particle bombardment with gene cassette</td>
<td>01-137-01p</td>
<td>October 2002</td>
<td>Selectable marker gene and a fragment of a superfluous gene</td>
<td>Yes, but sequence is not publicly available</td>
</tr>
<tr>
<td>63-1 Papaya</td>
<td>Particle bombardment with whole plasmid</td>
<td>96-051-01p</td>
<td>September 1996</td>
<td>Both lines included selectable marker genes and plasmid DNA sequences</td>
<td>n.d.</td>
</tr>
<tr>
<td>55-1 Papaya</td>
<td>Particle bombardment with whole plasmid</td>
<td>96-051-01p</td>
<td>September 1996</td>
<td>Both lines included selectable marker genes and plasmid DNA sequences</td>
<td>n.d.</td>
</tr>
<tr>
<td>Roundup Ready® Soybean 40-3-2</td>
<td>Particle bombardment with whole plasmid</td>
<td>93-258-01p</td>
<td>May 1994</td>
<td>Superfluous transgene sequences and unknown DNA sequences</td>
<td>n.d. (probable deletion and/or rearrangement at insertion site)</td>
</tr>
<tr>
<td>Maize YieldGard® MON810</td>
<td>Particle bombardment</td>
<td>96-017-01p</td>
<td>March 1996</td>
<td>none</td>
<td>n.d. (probable deletion and/or rearrangement at insertion site)</td>
</tr>
</tbody>
</table>
Unintended modifications of MON 810 maize

a) Loss of two fragments of the Bt (insect resistance) construct, Hernandez et al. (2003) leading to a second patent after discovery

b) Insertion of the construct in a relevant gene (ubiquitin ligase) and synthesis of completely new RNA products possibly leading to unknown proteins. Bogani et al. (2008)

c) Significant changes in the metabolic structure putatively affecting the nutritional value (Motto et al. 2006)
The production of fusion RNAs, putatively leading to new proteins, has been shown also in transgenic soybean. According to EFSA guidelines no analyses of the transcriptome through cDNA studies nor microarray studies on gene expression patterns in transgenic and so-called near isogenic lines are requested. Only computational studies on possible fusion proteins are required and expression profiles are limited to the inserted sequence thus disregarding the possible interactions with the pre-existing genetic context.
Differences between MON810 and control for more than 100 proteins. No proteome analysis is asked in EFSA guidelines.

Proteomics as a Complementary Tool for Identifying Unintended Side Effects Occurring in Transgenic Maize Seeds As a Result of Genetic Modifications

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Received August 6, 2007

To improve the probability of detecting unintended side effects during maize gene manipulations by bombardment, proteomics was used as an analytical tool complementary to the existing safety assessment techniques. Since seed proteome is highly dynamic, depending on the species variability and environmental influence, we analyzed the proteomic profiles of one transgenic maize variety (event MON 810) in two subsequent generations (T05 and T06) with their respective isogenic controls (WT05 and WT06). Thus, by comparing the proteomic profiles of WT05 with WT06 we could determine the environmental effects, while the comparison between WT06 and T06 seeds from plants grown under controlled conditions enabled us to investigate the effects of DNA manipulation. Finally, by comparison of T05 with T06 seed proteomes, it was possible to get some indications about similarities and differences between the adaptations of transgenic and isogenic plants to the same strictly controlled growth environment. Approximately 100 total proteins resulted differentially modulated in the expression level as a consequence of the environmental influence (WT06 vs WT05), whereas 43 proteins resulted up- or down-regulated in transgenic seeds with respect to their controls (T06 vs WT06), which could be specifically related to the insertion of a single gene into a maize genome by particle bombardment. Transgenic seeds responded differentially to the same environment as compared to their respective isogenic controls, as a result of the genome rearrangement derived from gene insertion.
A metabonomic study of transgenic maize (Zea mays) seeds revealed variations in osmolytes and branched amino acids

Cesare Manetti*, Cristiano Blanchetti, Lorena Casciani, Cecilia Castro, Maria Enrica Di Cocco, Alfredo Miccheli, Mario Motto and Filippo Conti

Table 3. ANOVA results for selected signals from control (c) and transgenic (t) maize seed spectra

<table>
<thead>
<tr>
<th>Metabolite (signal)</th>
<th>F-value$^a$</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate ($\beta$CH$_3$)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Ala ($\beta$CH$_3$)</td>
<td>14.6**</td>
<td>t $\ll$</td>
</tr>
<tr>
<td>$\alpha$-Glucose (C1H)</td>
<td>12.6**</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Asn ($\beta$CH$_2$)</td>
<td>18.6***</td>
<td>t $\ll$</td>
</tr>
<tr>
<td>$\beta$-Glucose (C1H)</td>
<td>17.2**</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Choline [N(CH$_3$)$_2$]</td>
<td>105.6***</td>
<td>t $\ll$</td>
</tr>
<tr>
<td>Dimethylamine (CH$_3$)</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Ferulic acid (HF)</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Formate (CH)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>GABA ($\alpha$CH$_2$)</td>
<td>28.8****</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Gln ($\beta$CH$_2$)</td>
<td>18.5***</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Glu ($\gamma$CH$_2$)</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>His (C2H, ring)</td>
<td>9.2**</td>
<td>t $\ll$</td>
</tr>
<tr>
<td>Ile ($\gamma$CH$_3$)</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Melibiose (Gal1H)</td>
<td>6.6*</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Pyruvate (CH$_2$)</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Succinate ($\alpha$-CH$_2$)</td>
<td>44.5***</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Sucrose (F1H)</td>
<td>7.1*</td>
<td>t $\gg$</td>
</tr>
<tr>
<td>Thr ($\gamma$CH$_3$)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Trigonelline (HA)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Tyr (C2, H6, ring)</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Val (CH$_3$)</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

$^a$**,**,**,** Significant at the .05, 0.01, and 0.001 probability levels, respectively.

Significant differences in 11 out of 22 compounds analysed between MON810 and control (50%). No metabolome analysis in EFSA guidelines)
Metabolome analysis with up to date methods is not required by EFSA procedures, research being limited to compositional studies only related to a small number of compounds. The criteria of MIAME (Minimum information about a microarray experiment) and MIAPE (Minimum information about a proteomics experiment) are not even mentioned in EFSA guidelines.
Significant differences in the peripheral immune response of rats fed with MON810 from control. No analysis of immune response in EFSA guidelines
Modifications in hepatocytes have been observed in long term experiments on rats fed with transgenic soybean. EFSA guidelines involve short term feeding trials on a limited number of rats.
At variance with EFSA suggestions data coming from long term trials in feeding experiments should be analysed with proper statistical techniques to obtain statistical significant meaning.

A Comparison of the Effects of Three GM Corn Varieties on Mammalian Health

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Abstract

We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been modified to be tolerant to the broad spectrum herbicide Roundup and thus contains residues of this formulation. MON 810 and MON 863 are engineered to synthesize two different Bt toxins used as insecticides. Approximately 60 different biochemical parameters were classified per organ and measured in serum and urine after 5 and 14 weeks of feeding. GM maize-fed rats were compared first to their respective isogenic or parental non-GM equivalent control groups. This was followed by comparison to six reference groups, which had consumed various other non-GM maize varieties. We applied nonparametric methods, including multiple pairwise comparisons with a False Discovery Rate approach. Principal Component Analysis allowed the investigation of scattering of different factors (sex, weeks of feeding, diet, dose and group). Our analysis clearly reveals for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also noticed in the heart, adrenal glands, spleen and haematopoietic system. We conclude that these data highlight signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM corn. In addition, unintended direct or indirect metabolic consequences of the genetic modification cannot be excluded.

Key words: GMO, toxicity, GM corn, rat, NK 603, MON 810, MON 863
Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi

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Key words: arbuscular mycorrhizal fungi, Bt toxin, environmental impact, plant defensins, root exudates of transgenic plants, test system

Abstract

We developed an experimental model system to monitor the impact of generically modified (GM) plants on arbuscular mycorrhizal (AM) fungi, a group of non-target soil microorganisms, fundamental for soil fertility and plant nutrition. The system allowed us to study the effects of root exudates of both commercial Bt corn and aubergine plants expressing Dm-AMP1 defensin on different stages of the life cycle of the AM fungal species G. mosseae. Root exudates of Bt 176 corn significantly reduced pre-symbiotic hyphal growth, compared to Bt 11 and non-transgenic plants. No differences were found in mycelial growth in the presence of Dm-AMP1 and control plant root exudates. Differential hyphal morphogenesis occurred irrespective of the plant line, suggesting that both exuded Bt toxin and defensin do not interfere with fungal host recognition mechanisms. Bt 176 affected the regular development of appressoria, 36% of which failed to produce viable infection pegs. Our experimental model system represents an easy assay for testing the impact of GM plants on non-target soil-borne AM fungi.

An analysis of the effect of transgenic plants on soil microflora and particularly on mycorrhizae-plants symbiosis have shown significant unintended affects but EFSA does not recommend this area of genotype-environment interactions.
An unintended effect: the pace of improvement of maize yield did not change after 1996, the year of introduction of GMOs into the market according to official USDA data.
The same data as in Fig 1 presented by Syngenta, one of the leading companies in the field showing a non existent “jump” in production since the introduction of GMOs in 1996.
D. INFORMATION RELATING TO THE GM PLANT

1. Description of the trait(s) and characteristics which have been introduced or modified

2. Information on the sequences actually inserted or deleted

3. Information on the expression of the insert

4. Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability

5. Genetic stability of the insert and phenotypic stability of the GM plant

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed
   7.1 Comparative assessment
   7.2 Production of material for comparative assessment
   7.3 Selection of material and compounds for analysis
   7.4 Agronomic traits
   7.5 Product Specification
   7.6 Effect of processing
   7.7 Anticipated intake/extent of use
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