Tailor-made plants using next generation molecular scissors

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Outline

- Why plant genome editing?
  - Plants for food, feed and fuel
    - Plants as production platforms
  - Off-target effects in plants
    - Regulatory aspects and commercialization
  - Challenges for plants
- Future developments
**Why do we need plant genome editing at all?**

- **Mutations** are the basis of evolution and biodiversity
- Are the essence of plant breeding
- Can arise from mistakes in the repair process of a DNA break
- 1930s ➔ radiations and chemicals to induce mutations (‘classical mutagenesis’)
  ~3,000 plants (wheat varieties, vegetables, fruit, rice, herbs…)
- 1970s ➔ first molecular scissors discovered (MN)
- Programmable site-specific nucleases:
  - 1996 ➔ ZFN
  - 2010 ➔ TALEN;
  - 2012 ➔ CRISPR/Cas9
Why do we need plant genome editing at all?

- To improve plants in a **more efficient** way
- To improve plants **faster**
- To improve plants in a **transgene-free** (non GMO ?) way
- To improve the way of making **transgenic**

Genome editing has been performed on crops such as barley, rice, tobacco, maize, wheat, potato, tomato, soybean, orange, grapevine...
genome editing can provide breeders with valuable tools for battling sustainability challenges

1) Point mutations/KO

- Increase resistance to pests ⇒ improve quality, improve yield, reduce costs, protect environment
  
  **Resistance to viruses** Cucumber vein yellowing virus, Zucchini yellow mosaic virus and Papaya ring spot virus through knock out of the cucumber eukaryotic translation initiation factor 4E (Chandrasekaran et al. 2016)

- Increase biomass, grain size, grain number ⇒ improve yield
  
  **Enhanced grain yield** in rice through knock out of four yield-related genes (Li et al. 2016)

- Increase crop adaptability to (changing) environmental conditions (draught, salt) ⇒ improve yield

- Increase the uptake of nutrients such as P and N ⇒ reduce fertilizers, reduce costs, protect environment
We depend on **agriculture** for **food**, **feed** and **fuel**

2) **Chromosomal rearrangements/** creating or breaking linkage of traits

3) **Cisgenesis**

![Image of apple and apple scab]

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It took 85 years to breed a tasty commercial apple containing the defense gene from an unappetizing relative that is resistant to apple scab.

4) **Trait stacking**

Combine multiple, independently segregating traits avoiding severe downstream breeding challenges and unrealistic timescales.
Plants can do much more: **molecular farming / metabolic engineering**

Pharmaceutical or technical proteins and metabolites can be produced in bulk in plants:

- **2G12 anti HIV antibody**
- **Elelyso® for Gaucher Disease**
- **Anti Ebola antibody cocktail ZMapp™**
Plants can do much more: **molecular farming / synthetic biology**

**How can genome editing be of use?**

- Promote targeted integration of transgenes
  - Plant chromosome
  - Donor
  - Targeted insertion

- Improve plants as production hosts for proteins
  - e.g. inactivating enzymes for plant-specific glycans

**Plant glycosylation vs. mammalian glycosylation**

\[ \beta_1,2 \text{-xylose} \quad \alpha_1,3 \text{-fucose} \]
Off-target effects are rare in plants

- Where reported, they tend to involve a minority of gRNAs
- Careful gRNA design can ensure specific targeting
- Likelihood of off-target mutations:
  
  Integrated DNA >> transient DNA > RNA > RNP complex

→ the frequency of off-target mutations is much lower than that of on-target mutations, allowing the recovery of solely on-target mutations in all experiments

→ much more precise than classical mutagenesis
GMO regulation depends on the country:

**Canada** has adopted a product-based regulation

the **U.S.** have a hybrid, case-dependent regulation

**Europe** has a process-based regulation

- EU overall has been indecisive to date about whether new techniques such as gene editing fall within the scope of its strict regulatory regime for GM products

- Social and political acceptance are critical

Classical Mutagenesis is excluded from the scope of the EU Directive 2001/18/EC
The US Department of Agriculture (USDA) will not regulate a mushroom that has been genetically modified with the gene-editing tool CRISPR-Cas9, the agency has confirmed. The long-awaited decision means that the mushroom can be cultivated and sold without passing through the agency’s regulatory process — making it the first CRISPR-edited organism to receive a greenlight from the US government.

"The research community will be very happy with the news," says Canisio Gao, a plant biologist at the Chinese Academy of Sciences Institute of Genetics and Developmental Biology in Beijing, who was not involved in developing the mushroom. "I am confident we'll see more gene-edited crops falling outside of regulatory authority."

Yongang Yang, a plant pathologist at Pennsylvania State University (Penn State) in University Park, engineered the fungus — the common white button mushroom (Agaricus bisporus) — to resist browning. The effect is achieved by targeting the family of genes that encodes polyphenoloxidase (PPO), an enzyme that causes browning. By deleting just a handful of base pairs in the mushroom’s genome...

USDA approval ➔ first CRISPR-edited organism approved in the US
### Regulatory aspects and commercialization

<table>
<thead>
<tr>
<th>Technology</th>
<th>Plant</th>
<th>Trait</th>
<th>Development phase</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ODM</strong></td>
<td>Canola</td>
<td>Sulfonylurea herbicide-tolerant</td>
<td>Launched in the US in 2015, expected in Canada in 2017 and in other major global markets in 2018</td>
<td>Cibus™</td>
</tr>
<tr>
<td></td>
<td>Flax</td>
<td>Glyphosate-tolerant</td>
<td>Expected launch in the US in 2019, and in Canada in 2020</td>
<td>Cibus™</td>
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<tr>
<td></td>
<td>Potato</td>
<td><em>P. infestans</em>-resistant</td>
<td>Launch expected first in the US in late 2019</td>
<td>Cibus™</td>
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<tr>
<td></td>
<td>Rice</td>
<td>Herbicide-tolerant</td>
<td>Launch expected first in the US end of the decade</td>
<td>Cibus™</td>
</tr>
<tr>
<td><strong>ZFN</strong></td>
<td>Maize</td>
<td>Herbicide resistance</td>
<td>Development phase</td>
<td>Dow AgroSciences</td>
</tr>
<tr>
<td><strong>TALEN</strong></td>
<td>Canola</td>
<td>Oil with lower levels of saturated fat</td>
<td>Development phase</td>
<td>Calyxt Inc.</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>Cold storable</td>
<td>First field trial completed in 2015</td>
<td>Calyxt Inc.</td>
</tr>
<tr>
<td></td>
<td>Soybean</td>
<td>High oleic acid and low linoleic acid content</td>
<td>Production of 30 tons in Argentina, launch expected in 2018</td>
<td>Calyxt Inc.</td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>Reduced gluten</td>
<td>Development phase</td>
<td>Calyxt Inc.</td>
</tr>
<tr>
<td><strong>CRISPR</strong></td>
<td>Maize</td>
<td>Drought-resistant</td>
<td>Launch expected in 5–10 years</td>
<td>DuPont Pioneer</td>
</tr>
<tr>
<td></td>
<td>Maize</td>
<td>Improved starch composition</td>
<td>Launch expected within 5 years</td>
<td>DuPont Pioneer</td>
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Challenges for plants

1) **Technical** problems:

- Efficient delivery (of RNPs) through the cell wall
- Understand and influence the DNA repair pathways

2) **‘Social’** problems:

- Regulatory issues and public acceptance (especially in Europe)
Future developments

- Discovery and optimization of other site specific nucleases
- Promiscuous protein + RNA combination
- Base editing enzymes

![Diagram of Cytidine to Uridine conversion with water and ammonium ions]
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