Tailor-made plants using next generation molecular scissors

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March 31st, 2017 GAFoE, Evendale, Ohio, USA

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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

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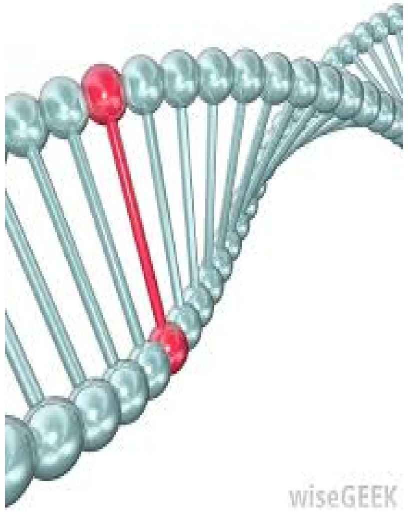
Why do we need plant genome editing at all?



- **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…

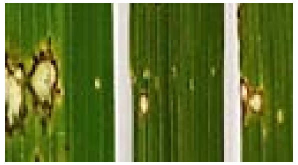
We depend on **agriculture** for **food**, **feed** and **fuel**

Genome editing can provide breeders with valuable tools for battling **sustainability challenges**

1) Point mutations/KO

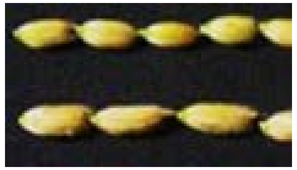
- Increase resistance to pests => *improve quality, improve yield, reduce costs, protect environment*

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)



Enhanced blast resistance in rice through mutation of the ERF transcription factor gene *OsERF922* (Wang *et al.* 2016)

- Increase biomass, grain size, grain number => *improve yield*



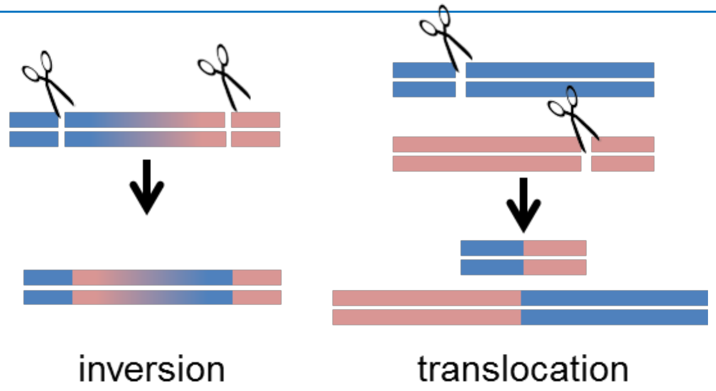
Enhanced grain yield in rice through knock out of four yield-related genes (Li *et al.* 2016)

- 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

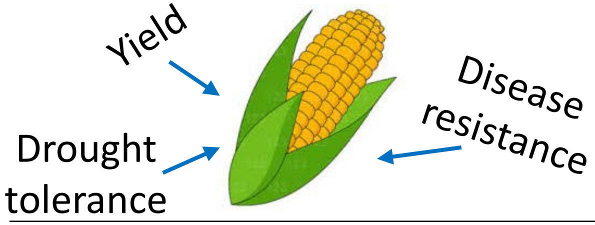


3) Cisgenesis


doi:10.1038/500389a

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

Pharma Planta

Elelyso® for Gaucher Disease

PROTALIX Biotherapeutics

Anti Ebola antibody cocktail ***ZMapp***™

Mapp Biopharmaceutical

DOI: 10.13031/2013.26854

Plants can do much more: **molecular farming / synthetic biology**

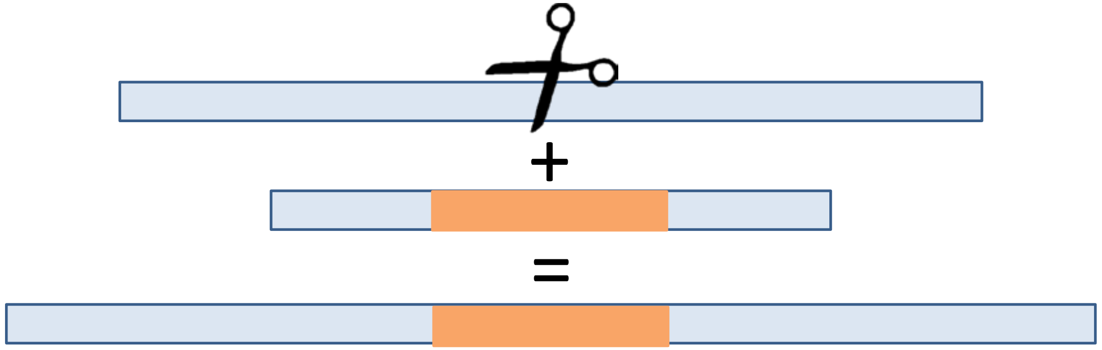
How can genome editing be of use?

➔ Promote targeted integration of transgenes

Plant chromosome

Donor

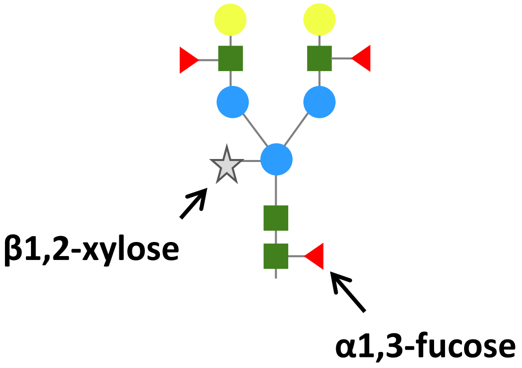
Targeted insertion



➔ Improve plants as production hosts for proteins

e.g. inactivating enzymes for plant-specific glycans

Plant glycosylation vs. **mammalian** glycosylation



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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

Regulatory aspects and commercialization

➔ **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

Regulatory aspects and commercialization

nature 21 APRIL 2016 | VOL 532 | NATURE | 293

BIOTECHNOLOGY

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered using CRISPR–Cas9 can be cultivated and sold without oversight.

BY EMILY WALTZ

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 green light from the US government.

"The research community will be very happy with the news," says Caixia 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."

Yinong 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 polyphenol oxidase (PPO), an enzyme that causes browning. By deleting just a handful of base pairs in the mushroom's genome

STUART MCCALL/GETTY

The common white button mushroom (*Agaricus bisporus*) has been modified to resist browning.

USDA approval➔ first CRISPR-edited organism approved in the US

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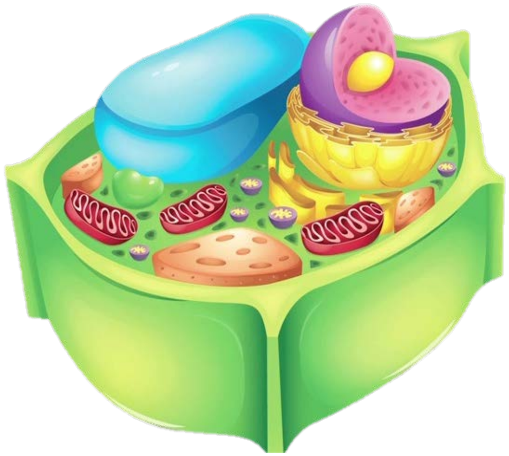
Regulatory aspects and commercialization

[1] Based on publicly available information

Technology[1]	Plant[1]	Trait[1]	Development phase[1]	
ODM	Canola	Sulfonylurea herbicide-tolerant	**Launched in the US in 2015**, expected in Canada in 2017 and in other major global markets in 2018	Cibus™
	Flax	Glyphosate-tolerant	Expected launch in the US in 2019, and in Canada in 2020	
	Potato	*P. infestans*-resistant	Launch expected first in the US in late 2019	
	Rice	Herbicide-tolerant	Launch expected first in the US end of the decade	
ZFN	Maize	Herbicide resistance	Development phase	Dow AgroSciences
TALEN	Canola	Oil with lower levels of saturated fat	Development phase	Calyxt Inc.
	Potato	Cold storable	First field trial completed in 2015	
	Soybean	High oleic acid and low linoleic acid content	Production of 30 tons in Argentina, launch expected in 2018	
	Wheat	Reduced gluten	Development phase	
CRISPR	Maize	Drought-resistant	Launch expected in 5–10 years	DuPont Pioneer
	Maize	Improved starch composition	**Launch expected within 5 years**	

Challenges for plants

1) **Technical** problems:



http://study.com

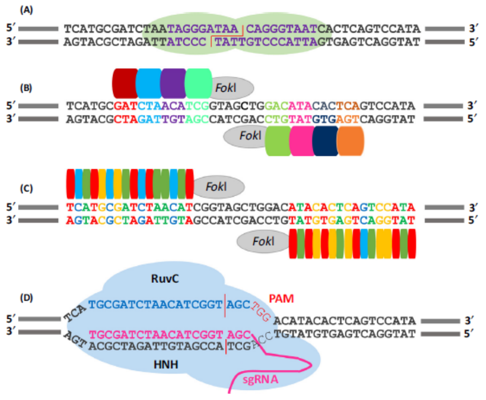
→ Efficient delivery (of RNPs) through the cell wall

→ Understand and influence the DNA repair pathways

2) **‘Social’** problems:

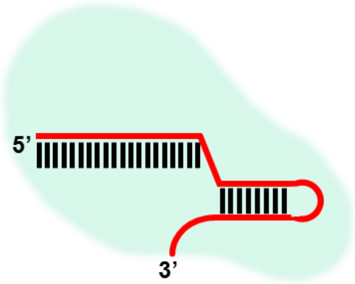
→ Regulatory issues and public acceptance (especially in Europe)

Future developments

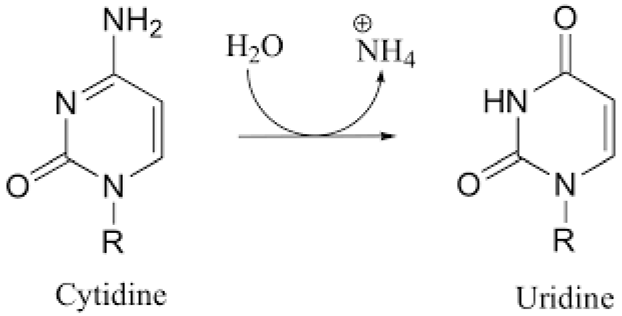


➔ Discovery and optimization of other site specific nucleases

➔ Promiscuous protein + RNA combination



➔ Base editing enzymes



Acknowledgments

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RHEINISCH-WESTFÄLISCHE TECHNISCHE HOCHSCHULE AACHEN

Prof. Dr. Rainer Fischer
Julia Zischewski
Markus Sack
Dr. Sruthy Augustine
Dr. Flora Schuster

Fraunhofer IME

Prof. Dr. Rainer Fischer
Prof. Dr. Stefan Schillberg

Universitat de Lleida

Prof. Dr. Paul Christou
Can Baysal
Dr. Changfu Zhu