CRISPR-Cas9 Genome Editing

New Era of Agricultural Biotechnology

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Precise Gene Editing in Plants

CRISPR offers an easy, exact way to alter genes to create traits such as disease resistance and drought tolerance.

Availability: 5-10 years
Outline

• The CRISPR-Cas9 System

• Implementations and Beyond

• Barley Genome Editing

• Outlook and Challenges
What is genome editing?

- A genetic engineering approach in which DNA is inserted, removed or replaced in the genome in a **precise** manner.
- Programmable nucleases create site-specific double-strand breaks (DSBs) at **desired** locations in the genome.
- The induced DSBs are repaired through non-homologous end-joining (NHEJ) or homologous recombination (HR), resulting in **targeted** mutations ('edits').

*Make nearly any desirable modification!*
## Comparison of breeding technologies

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Physicochemical (e.g., EMS, gamma, or X-ray)</th>
<th>Biological-Transgenics (e.g., Agro or gene gun)</th>
<th>Biological-Genome editing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of genetic variation</td>
<td>Substitutions and deletions</td>
<td>Insertions</td>
<td>Substitutions, deletions and insertions</td>
</tr>
<tr>
<td></td>
<td>Loss of function mainly</td>
<td>Loss of function and gain of function</td>
<td>Loss of function and gain of function</td>
</tr>
<tr>
<td>Advantages</td>
<td>Unnecessary of knowing gene function or sequence</td>
<td>Random insertion of genes with known functions into host</td>
<td>Gene specific mutation</td>
</tr>
<tr>
<td></td>
<td>Easy production of random mutation</td>
<td>Efficient creation of plants with desirable traits</td>
<td>Efficient production of desirable mutation</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Inefficient screening of desirable traits</td>
<td>Necessity of knowing gene function and sequences</td>
<td>Necessity of knowing gene function and sequences</td>
</tr>
<tr>
<td></td>
<td>Non specific mutation</td>
<td>Prerequisite of efficient genetic transformation</td>
<td>Prerequisite of efficient genetic transformation</td>
</tr>
<tr>
<td>Other features</td>
<td>Non transgenic process and traits LiLy Lily</td>
<td>Transgenic process and traits</td>
<td>Transgenic process but non transgenic traits</td>
</tr>
</tbody>
</table>

Xiong et al. Horticulture Research, 2015
CRISPR/Cas9—a clever immune system

The type II CRISPR locus from *S. pyogenes* SF370: Most well-understood and simplest
Plant genome editing

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What can CRISPR-Cas9 do?

• Basic research
  • Generate alleles and gene mutations
  • Analysis of linked/lethal genes

• Agricultural productivity
  • Non-transgenic approaches for crop breeding
  • Genome engineering of plants and animals

• Biotechnology
  • Ecological control of vectors transmitting diseases
  • Synthetic biology

• Disease modelling
  • Gene therapy
  • Fix specific cell-types
Crops modified with CRISPR technology

<table>
<thead>
<tr>
<th>CROPS</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Liang et al. 2014</td>
</tr>
<tr>
<td>Rice</td>
<td>Belhaj et al. 2013</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Jiang et al. 2013</td>
</tr>
<tr>
<td>Sweet orange</td>
<td>Jia and Wang 2014</td>
</tr>
<tr>
<td>Tobacco</td>
<td>Belhaj et al. 2013</td>
</tr>
<tr>
<td>Wheat</td>
<td>Wang Y. et al. 2014</td>
</tr>
<tr>
<td>Soybean</td>
<td>Cai et al., 2015</td>
</tr>
<tr>
<td>Potato</td>
<td>Wang S. et al., 2015</td>
</tr>
<tr>
<td>Barley</td>
<td>Lawrenson et al., 2015</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Lawrenson et al., 2015</td>
</tr>
</tbody>
</table>
Simultaneous editing of three homoeoalleles in hexaploid bread wheat

Wang et al., Nature Biotechnology, 2014
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Targeting barley PM19 gene

HvPM19: ABA-inducible plasma membrane protein

sgRNA Design

Lawrenson et al., Genome Biology, 2015

Germline transmission of Cas9 induced mutations in representative progenies

Lawrenson et al., Genome Biology, 2015
A key gene controlling multiple traits

Gene expression

Plant height

Yield

Heading date

Kernel weight

Plumpness
The “edited” barley gene for yield

Multiple alleles of the gene through mutation

Gene cloning and plasmid construction

5’...CAGAGTACTGAGGAGAGAACAACGTGCGATGATGATATTGCAGGAGGGGTGTACCGAGGATCG
CGGCAAGATGAGGAGCTGTCGCTGAGGATCATGGAGGCTGTCGCTGAGCTGAGCCTGGGGCGTGG
GAAGCGCGGTACTACCAGGGACTTCTTCTCGCGGACAGCAGCTCCATCATGCGGTGCAACTACTACC
CGCCGTGCCCGGGAGGCCGAGCGCAGCGTGGGCACGGGCGCCGCACTCGGACGCCCCACGCGCTCA
CCATCCTCCTCCAGGACGACGTGGGGCGGCTGAGGTCTCTCGTGCAGCGCAGCTGGGCGCCG
CCGCCCCGTCCCCGCGGCGCCATGGCATACATCCCGCGACACCTCATGGAATTACATTACAGTAGTA
TTAGCGTTGCTTTCTCGTGAATAGCTGGGACACATAACGTAAGTAACG...3’

Binary vector 14, 451 bp
Barley genetic transformation

Barley donor plants continuously grown for immature embryos

Agrobacterium-mediated barley genetic transformation

- Genotyping and identifying the inheritance of mutations;
- Phototyping the agronomic traits in progenies.

Isolated embryos

Callus induction  Selection  Plant regeneration  Genotyping
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Genome editing - Comes of age

Million genomes projects

Assembled WGS from 87 species

Omics, GWAS, QTL mapping

SPEED/Price

CRISPR/Cas9

Accuracy/Specificity
Box 1. A spotlight on two ambitious synthetic biology projects

Examples of plant synthetic biology projects include the nitrogen-fixing cereals project (Figure 1A) and the C₄ rice project (Figure 1B).

By engineering cereals to uptake atmospheric nitrogen, there will be a reduced dependency on inorganic fertilizers. There are two possible approaches for modifying cereals to uptake atmospheric nitrogen: transfer the nodulation signaling pathway from legumes to promote root nodule symbiosis with *Rhizobium* bacteria, or engineer the nitrogenase enzyme to function in plant cells.

Engineering the C₄ photosynthesis pathway into C₃ rice promises to increase yield. One approach to engineering this pathway in rice is to convert the single-cell C₃ cycle into a two-celled C₄ cycle. In this case the initial carbon fixation is catalyzed within mesophyll cells by phosphoenolpyruvate carboxylase (PEPC) forming the four-carbon oxaloacetate from bicarbonate and PEP. Oxaloacetate is then metabolized into malate, and the four-carbon acid diffuses into the bundle sheath cell. There, the four-carbon acid is decarboxylated to provide increased concentrations of carbon dioxide to RuBisCO, which is confined in bundle sheath cells.

Figure 1. Two examples of synthetic biology projects aimed at generating nitrogen-fixing cereals or C₄ photosynthetic rice.
**Swedish Board of Agriculture: Some CRISPR-Cas9 edited plants not GMO**

The Swedish Board of Agriculture has, after questions from researchers in Umeå and Uppsala in Sweden, confirmed the interpretation that some plants in which the genome has been edited using the CRISPR-Cas9 technology do not fall under the European GMO definition. The case brought forward by the researchers and the interpretation by the Board of Agriculture are covered in a news article and an editorial in a recent issue of Nature (15 December 2015).

CRISPR-Cas9 is a technique, which allows scientists to make small edits in the genetic material of an organism, edits that can also occur naturally. Instead of waiting for such edits to occur by natural recombination, they can now be deliberately introduced in a targeted and precise manner. CRISPR-Cas9 can thus be used in many ways in plant science and breeding.

**Gene-edited CRISPR mushroom escapes US regulation**

A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight.

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

**CRISPR corn: DuPont's non-transgenic drought-tolerant gene edited corn could be on sale soon**

Alexandra Ossola | September 8, 2016 | Popular Science

Researchers at DuPont Pioneer... have published a study about a strain of corn engineered with CRISPR to be more resistant to drought. Once it receives government approval, this could soon be the first-ever CRISPR-modified crop to go on sale.

The technique outlined in the study, published August...
Take home message

- Genome-editing offers technical advantage over the traditional GMO
- CRISPR-Cas9 has been successfully used for major crops (wheat, maize, rice, barley)
- The products may not be classified as GMO
- CRISPR-Cas9 platform is established in MU
- Proof of concept for barley yield and quality traits
Thank you!

- Prof. Chengdao Li, Director, Western Barley Genetics Alliance