Gene editing for accelerated breeding in cereals

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Gene editing for accelerated breeding

Modified from Wulff and Dhugga, Science (2018)
Challenge in recovering elite genetic background via backcrossing

*it is not only the time*

<table>
<thead>
<tr>
<th>Generation</th>
<th>Genome (%) recurrent parent</th>
<th>Genome (%) donor parent</th>
<th>Donor genes (maize)</th>
<th>Donor genes (wheat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>50.0</td>
<td>50.0</td>
<td>20,000</td>
<td>50,000</td>
</tr>
<tr>
<td>BC1</td>
<td>75.0</td>
<td>25.0</td>
<td>10,000</td>
<td>25,000</td>
</tr>
<tr>
<td>BC2</td>
<td>87.5</td>
<td>12.5</td>
<td>5,000</td>
<td>12,500</td>
</tr>
<tr>
<td>BC3</td>
<td>93.8</td>
<td>6.2</td>
<td>2,500</td>
<td>6,250</td>
</tr>
<tr>
<td>BC4</td>
<td>96.9</td>
<td>3.1</td>
<td>1,250</td>
<td>3,125</td>
</tr>
</tbody>
</table>

*Proportion of recurrent genome = \((2^{n+1}-1)/2^{n+1}\)*
Three scenarios for gene editing: SDN1, SDN2, and SDN3

Figure 1. Different site-directed nuclease (SDN) techniques (SDN-1, 2, and 3). An SDN complex is shown at the top in association with the target sequence. The repair can take place via nonhomologous end-joining (NHEJ) or homologous recombination (HR) using the donor DNA. SDN-1 can result in site-specific random mutations by NHEJ. In SDN-2, a homologous donor DNA is used to induce specific nucleotide sequence changes by HR. In SDN-3 DNA is integrated in the plant genome via HR.
CRISPR-edited waxy trait demonstrates rapid product development (Corteva Agriscience)

- Editing: May 2015
- ~42 months
- Product development complete fall 2018
- Breeding
- Transgenic
- 1st commercial sales in year 9
- 1st commercial sales in year 13-20
Traits for gene alteration at CIMMYT

- **Maize**
  - Resistance to maize lethal necrosis (MLN)
  - Biofortification
    - Increase provitamin A by down-regulating CCD genes
    - Fe and Zn availability via phytate downregulation

- **Wheat**
  - Disease resistance
    - Leaf rust (*Lr34, Lr67*)
    - Powdery mildew (*MLO*)
  - Plant height reduction by alternative mechanisms from Rht genes
  - Biofortification
    - Phytate downregulation for increased Fe and Zn availability
Genotypes resistant (L) or susceptible (R) to MLN

*Naivasha, Kenya*
When a drought-tolerant commercial hybrid becomes susceptible to MLN

- Commercial hybrid grown in Kenya and Uganda
- High yielding under drought and optimal conditions
- Turned out to be susceptible to MLN after the disease emerged

Kiboko: No MLN pressure

Naivasha: Artificial MLN inoculation

Beyene, Olsen

CML312/CML395//CML566
An exotic line is resistant to MLN
MLN resistance (MLN$_R$) maps to a single QTL
Kenya

Three populations under field conditions

Mike Olsen
**MLN\textsubscript{R} locus alone explains half of the variation for MLN resistance**

<table>
<thead>
<tr>
<th>Disease score (1-5, R-S)</th>
<th>CML494</th>
<th>CZL03018</th>
<th>CML545</th>
<th>CZL00025</th>
<th>SUSCEP</th>
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</thead>
<tbody>
<tr>
<td>Parent in cross</td>
<td>CML068</td>
<td>KS23-6</td>
<td>KS23-5</td>
<td>KS23-5</td>
<td>KS23</td>
</tr>
</tbody>
</table>

Parents in cross (KS23-6 as common donor)

Olsen
Effect of $\text{MLN}_R$ allele (KS23-6) on resistance


- KS23-6 allele
+ KS23-6 allele

Mike Olsen
Transformation of CIMMYT lines

*These four lines form two, 3-way cross commercial hybrids*

- Obtained more than 100 T0 plants for each line
- Transformation frequency: 100%
MLO resistance: inactivate all three copies (A, B, and D)

Sequence identity of:
TaMlo-A1 and B1 = 95%
TaMlo-A1 and D1 = 96%
TaMlo-B1 and D1 = 97%

>3 kb
<table>
<thead>
<tr>
<th>CIMMYT</th>
<th>Corteva Agriscience</th>
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<tbody>
<tr>
<td>Yoseph Beyene</td>
<td>Mark Jung</td>
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<tr>
<td>Mike Olsen</td>
<td>Alyssa DeLeon</td>
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<tr>
<td>Veronica Ogugo</td>
<td>Bob Meeley</td>
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<td>L.M. Suresh</td>
<td>Kevin Simcox</td>
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<td>Jeff Farrell</td>
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<td>Todd Jones</td>
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<td>Kay Snopek</td>
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<td>Emily Wu</td>
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<td>Bill Gordon-Kamm</td>
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<td>Shawn Thatcher</td>
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<td>Barbara Mazur</td>
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<td>Neal Gutterson</td>
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Funding: CRP-Maize, CRP-Wheat, and Bill & Melinda Gates Foundation
Accelerated breeding

• Reconstitution of original genetic background after backcrossing is time consuming yet suffers from linkage drag.

• Maize hybrids in Africa have long lifespans, lasting decades. When popular hybrids go out of production, for example, because of disease susceptibility, smallholder farmers encounter major disruptions.

• Corteva has revolutionized genetic transformation so the tropical maize lines from Africa can be directly edited.

• Edit target gene directly in elite lines.

• Future edits could be stacked onto the previous one.

• These steps will save years worth of time and eliminate linkage drag.

• Significantly contribute toward alleviating poverty and hunger.