Zinc finger nuclease (ZFN)-based mutagenesis of floral genes in poplar

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Goal
- Use ZFNs to mutate LEAFY and AGAMOUS orthologs in poplars – to study the efficiency of ZFN-induced mutagenesis
- Create poplars that do not flower or have bisexually sterile flowers – to prevent gene flow

Mechanism of ZFN action
- Zinc finger protein and FokI fusion
- Work in pairs
- Generate double stranded breaks
- Lead to indel mutations

Four ZFN pairs were created

Heat-inducible ZFN expression and GFP marker

ZFNs were hot in the old days of genome editing!

PLANT BIOTECHNOLOGY
Zinc fingers on target
Matthew H. Porteus
The existing methods of creating genetically modified plants are inefficient and imprecise. Zinc-finger technology offers the prospect of opening up a swifter and more exact route for crop improvement.

NEWS & VIEWS
SET UP FOR SUCCESS: May 2016

Details see poster # 1187
ZFNs depress transformation rate

<table>
<thead>
<tr>
<th>Construct</th>
<th>No. of transformed explants</th>
<th>No. of confirmed ZFN transformants</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZFN800(PtAGs)</td>
<td>717 (F)</td>
<td>353 (M)</td>
</tr>
<tr>
<td>ZFN801(PtAGs)</td>
<td>2,140</td>
<td>393</td>
</tr>
<tr>
<td>ZFN802 (PtLFY)</td>
<td>2,231</td>
<td>0</td>
</tr>
<tr>
<td>ZFN803 (PtLFY)</td>
<td>3,431</td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>21,698</td>
<td>393</td>
</tr>
</tbody>
</table>

- Transformation rate of 1.8% with ZFN constructs
- Transformation rate of 7.7% with control (HSP:GFP)

Two mutants with mutated PtAG2 were detected with high resolution melting (HRM) analysis

- HRM – a PCR-based method for detecting DNA sequence polymorphisms or mutations
- Detects variants based on Tm values and the shape of the melt curves

TOPO cloning and sequencing revealed the nature of the mutation

<table>
<thead>
<tr>
<th>Gene</th>
<th>WT type</th>
<th>Mutant type</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZFN800</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
</tr>
<tr>
<td>ZFN801</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
</tr>
<tr>
<td>ZFN802</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
</tr>
<tr>
<td>ZFN803</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
<td>TCTCCCTCAGAAAGCTGGAAGGAAATGTTGCACTAGGAATGGAACACAC</td>
</tr>
</tbody>
</table>

- Both mutants contain deletions in one allele of PtAG2
- Pooling all data, overall mutation rate of 0.3% per allele per insertion event
- Unlikely to observe sterile flowers, as PtAG1 heterozygote likely to retain its function

Conclusions

- Modest mutation rate
- Only one of four ZFNs tested induced any mutations
  - Though several dozen had been tested in vitro mouse/yeast system
- Reduced recovery of transgenic plants, as a result of deleterious effects of ZFNs and heat shock treatment
  - Challenging to generate population of adequate size to obtain knock-outs
- Much development and optimization needed
  - Efficiency of newer CRISPR methods may obviate
  - See talk and poster by Elorriaga et al.

Welcome to visit poster # 1187 for details!

Acknowledgments

Amy L. Klocko  Michael Dow  Cathleen Ma  Vindhya Amarasinghe  Steven H. Strauss

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Dr. John Finer of Ohio State provided the GFP marker gene