Functional genomics of poplar bioenergy phenotypes using a unique dosage variants population

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Poplar trees, a promising feedstock for biofuel

A useful feedstock
• One of the fastest growing tree in temperate regions
• High content of cellulose in wood
• Hybrid vigor and clonal propagation
• Grown in short rotation coppice systems

A model for forest tree species
• Full genome sequence assembly of Populus trichocarpa (Tuskan et al., 2006)

Gene Dosage and Ploidy Changes are Associated with Transgressive Phenotypes in Many Crops

Challenges in poplar breeding program
• Drought resistance
• High yield/coppicing response
• Suitable wood chemistry (high cellulose/low lignin content)

A powerful tool to investigate the genomics of Populus for biofuel production

• Creation of a dosage variants population in aneuploid Populus
  • >800 lines available in F1
• Gamma irradiation (100 grays) of pollen creates chromosomal breaks
• Karyotyping by sequencing reveals chromosomal composition

Gamma-irradiation results in insertions and deletions

- 800 genotypes available
- 50% carry lesions
  75% deletions / 25% insertions
Triplotids adds subtle variation to the dosage ratios

Gamma-irradiation results in insertions and deletions

Tree phenotyping in the field
- Planted in triplicate in the Institute of Forest Genetics, California
- About 600 irradiated genotypes (100 grays), and 56 non irradiated control genotypes

<table>
<thead>
<tr>
<th>Traits recorded</th>
<th>To be recorded next</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf development</td>
<td>Leaf phenology/senescence, leaf specific area, leaf color, petiole length, chlorophyll content, stomata density</td>
</tr>
<tr>
<td>Biomass production</td>
<td>Tree height, diameter, number/size of the branches, internode length, growth rate</td>
</tr>
<tr>
<td>Tree architecture</td>
<td>Angle of the branches, length, density</td>
</tr>
<tr>
<td>Wood characteristics</td>
<td>Fiber length, wood specific gravity, cellulose content, etc</td>
</tr>
</tbody>
</table>

Drought resistance screening

Pot experiment
- 10 liters pots
- About 120 genotypes in triplicate
- Presence of a great diversity of leaf morphologies
- Application of a moderate stress for a month + well watered control plants

Evaluation:
- Biomass production
- Leaf chlorosis, wilting, senescence, chlorophyll content
- Leaf temperature
- Relative leaf water content
- Infrared spectrometry indexes
- Stomata density

Gravibending response in the mutant population

In pots
- Screening of about 90 genotypes in triplicate
- Plants placed horizontal for one month
- Measurement of the height of the apex of curvature
- Wood sampling

G-layer formation in the tension wood
Gravibending response in the mutant population

Contrasting gravibending response and amount of tension wood produced

Tension wood characteristics
• Faster growth – cell division rate
• Longer fiber with extra gelatinous layer (G-layer)
• High cellulose/low lignin content

Changes in the patterning and morphology of the vessel elements

In the mutant
Most of the vessels elements are in groups >3
(up to 32 vessels in one group)
Vessels are 3.5 times narrower and contain more perforations

A tool for reverse and forward genetics

• Opportunities for reverse genetics

Example: influence of indels on a TAC1 poplar paralog (Chr14 at 8.02 Mbp)

<table>
<thead>
<tr>
<th>Insertion in a 2n and 3n background</th>
<th>Deletion in a 3n background</th>
<th>Deletion in a 2n background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal to upright branches 4 genotypes</td>
<td>Upright branches – pillar tree 2 genotypes</td>
<td>Horizontal, weepy branches 4 genotypes</td>
</tr>
</tbody>
</table>

A tool for reverse and forward genetics

• Opportunities for forward genetics: dosage QTLs

Characterization of the relationships between quantitative phenotypes and dosage of specific genomic regions
Correlation analysis between the phenotypic data and bins along the chromosomes containing insertion and deletions:
- Detection of dosage sensitive genes only
- Possibility of trans-acting elements

Transcriptomic data analysis

• Investigate the regulatory consequence of dosage variations on gene expression

<table>
<thead>
<tr>
<th>Insertion</th>
<th>Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>[123456]</td>
<td>[01]</td>
</tr>
<tr>
<td>Hybrid with indel mutations</td>
<td></td>
</tr>
</tbody>
</table>

• Investigate the altered connectivity in transcriptomic networks induced by indels and the impact on phenotypes

Conclusion

Our dosage mutant population of Populus:
• represents a new resource of important phenotypic variants for poplar

• Constitutes a unique resource for tree genomics:
  – Tool for reverse and forward genetics
  – Is instrumental in investigating the relationships between phenotype, gene dosage and gene expression
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