Profiling of genes involved in the regeneration of secondary vascular system in poplar

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Wood formation

Diagram A: Detailed view of wood structure.

Diagram B: Schematic representation of wood formation:
- Cell division
- Expansion
- Elongation
- Cell wall layers
- Cellulose
- Pectins
- Hemicelluloses
- Lignin
- Monolignols

Phloem sections labeled A-E.
Background

- EST database: over 200,000
- Microarray analysis of gene expression in cambium zone
- Poplar genomic sequence
- Too little data on proteomics
- Thousands of genes expressed in wood forming tissues
- But their function in terms of development?
Establish of the wood regeneration system
Advantage of this system

• Can mimic (within one month)
  – Initiation of cambium
  – Differentiation of cambial cells
  – Development of xylem

• Obtain the materials for analysis by
  – Proteomics
  – Microarray
2-D Electrophoresis

Regeration stages
A: 6 d
B: 10 d
C: 14 d
D: 18 d
E: 22 d
MS Analysis

In total, 258 PMFs obtained

PMF of Spot106 from 22 days after

PMF of Spot108 from 22 days after
Microarray

- Images
  - A: Day10th VS Day 6th, 10G+6R;
  - B: Day10th VS Day 6th, 10R+6G;
  - C: Day10th VS Day 6th, enlarged B;
  - D: Day14th VS Day 12th, 14G+12R;
  - E: Day14th VS Day 12th, 14R+12G;
  - F: Day14th VS Day 12th, enlarged E
Great change of gene expression during the regeneration
Functional classification

A

No hit (39, 17.5%)
Signaling (18, 8.1%)
Transcription factor (16, 7.2%)
Cell wall (25, 11.2%)
Cell cycle (2, 0.9%)
Cytoskeleton (2, 0.9%)
Photosynthesis (4, 1.8%)
Metabolism (23, 10.3%)
Unknown (53, 23.8%)
Other (41, 18.4%)

B

Bar chart showing frequency of different functional classifications.
Expression by real-time PCR
How to identify the key genes?
Arabidopsis “wood”

(Chaffey, 2002)
### Arabidopsis Mutants

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Phenotypic change of Arabidopsis mutant
Arabidopsis mutants
Mutant

Widetype

SK10
Specific expression in vascular tissues

24 h

36 h
Specific expression in vascular system
Effect on flower development
Effect on the cell wall

5-10 M

5-12 M

5-14 M

5-17 M

WT
Up- and down-regulation of the candidate genes
Auxin related genes

| P49678     | Auxin-responsive protein | Arabidopsis thaliana | P |
| P33077     | Auxin-responsive protein IAA4 | Arabidopsis thaliana | P |
| P49678     | Auxin-responsive protein IAA2 | Arabidopsis thaliana | P |
| Q03663     | Auxin-induced protein PGNT35/PCNT111 | Nicotiana tabacum | P |
| gi|42569975 | ARF11 (AUXIN RESPONSE FACTOR 11) | Arabidopsis thaliana | P |
| gi|45503975 | Putative small auxin up RNA | Zea mays | P |
| gi|46576649 | Putative auxin response factor 13 | Arabidopsis thaliana | P |
| gi|52077231 | Auxin responsive protein-like | Oryza sativa | P |
| gi|34909612 | Putative auxin response transcription factor | Oryza sativa | P |
| gi|2462761 | Highly similar to auxin-induced protein (aldo/keto reductase family) | Arabidopsis thaliana | P |
| gi|77551195 | Auxin response factor, putative | Oryza sativa | P |
| gi|8778254 | Auxin response factor | Arabidopsis thaliana | P |
| gi|77552476 | Auxin-induced beta-glucosidase, putative | Oryza sativa | P |
| At4g25570 | Auxin induced Protein | Populus trichocarpa | P |
| At1g78380 | Auxin induced glutathione S-transferase | Populus trichocarpa | P |
| At3g15540, | Early auxin-induced protein IAA19 | Arabidopsis thaliana | M |
| At4g28640, | Auxin-induced protein IAA11 | Arabidopsis thaliana | M |
| NM_116532 | Auxin binding protein 1 | Arabidopsis thaliana | M |
*In situ RT-PCR*
Transformation of poplar with *PtABP1*
Expression of ABP1 in non- and transgenic plants

![Graph showing expression levels of ABP1 in different conditions.](image)
Increased cell size

Decreased cell wall

![Graphs showing increased cell size and decreased cell wall thickness.](image-url)
Early flowing of the over-expression tobacco line
Early initiating SVS development

Control

Transgenic
Early ending the SVS development
Summary

• SVS regeneration system
  – Cambium formation, differentiation and xylem development
• Thousands of genes involved in wood formation
• Candidate genes selected
• Characterization of role of these genes
  – Mutants
  – Transgenic approach
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