Possibilities of somatic embryogenesis for production of Scots pine trees with improved heartwood quality

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Aims of the study:

- Breeding SE lines from donor trees with known phenolic compound concentration
- Study the factors affect EC initiation
- Testing the concentration of phenolic compounds in the ECs
- Study the phenolic compounds in fungal resistance
Scots pine heartwood

- Scots pine heartwood is dead and extractive-rich tissue, which contains e.g. stilbenes pinosylvin (PS) and pinosylvin monomethyl ether (PSM)
- PS and PSM have inhibitory effects on the fungal growth, the higher concentration giving the higher effect
- PS and PSM concentrations in heartwood vary among trees and are highly inherited
- PS and PSM are phytotoxic compounds, and their biosynthesis in living tissues can be induced by biotic and abiotic stresses in the nature
**Phenolic concentrations of the donor trees**

<table>
<thead>
<tr>
<th>Phenolics level</th>
<th>HW</th>
<th>SW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS (mg/g)</td>
<td>PSM (mg/g)</td>
</tr>
<tr>
<td>K912</td>
<td>23,173</td>
<td>18,423</td>
</tr>
<tr>
<td>K836</td>
<td>14,477</td>
<td>12,141</td>
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<tr>
<td>K699</td>
<td>8,685</td>
<td>5,790</td>
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<tr>
<td>K927</td>
<td>7,524</td>
<td>10,042</td>
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<tr>
<td>K803</td>
<td>7,203</td>
<td>4,166</td>
</tr>
<tr>
<td>K917</td>
<td>5,693</td>
<td>5,280</td>
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</table>
We tested:

• Effect of the donor trees from both the open-pollinated donor trees in 2011 and by controlled crossings of the donors in 2012 and in 2013

• Effect of phenolic concentration in explants on ECs initiation

• Phenolic concentration in ECs and in emblings
Propagation of Scots pine from the known mother trees

- In 2011: **80** lines were initiated from the open pollinated donor trees.

- In 2012 & 2013: **128** lines initiated from the control-pollinated donor trees.

- Figures show the number of the initiation lines in both pollination conditions.
Stilbene concentrations in ECs & embryo production from the open pollinated trees

- Stilbene concentration in ECs varied from each genotypes
- The ECs with higher embryo production capacity contained less concentration of stilbenes
Fungal inhibition test using the ECs with high/low stilbenes

Resistance of stilbenes to *P. placenta*

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Low</th>
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<tbody>
<tr>
<td>467</td>
<td>0,5</td>
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<tr>
<td>486</td>
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<tr>
<td>759</td>
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<tr>
<td>1076</td>
<td>2,0</td>
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</tr>
<tr>
<td>515</td>
<td>2,5</td>
<td>2,0</td>
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<td>1036</td>
<td>1,0</td>
<td>0,5</td>
</tr>
<tr>
<td>1521</td>
<td>0,5</td>
<td>0,0</td>
</tr>
</tbody>
</table>

Chitin content (mg/g dryW)
STL gene in response to fungi treatments

- STS gene was induced significantly after 6 days in the line 1982, while not in the line 448.

- However, pathogenic fungus *H. annosum* could induce STS in the line 448 at 3 days.
Stilbene concentration were analyzed by HPLC after 6 days treatment.
STS gene expressed in Ecs, needles, roots and stems after UVC radiation
Stilbenes in response to UVC radiation

- Stilbene concentration varied from genotypes, organs and responding to UVC radiation;

- Pinosylvin in needles was induced by UVC radiation in the lines 448 and 2212.
Conclusions:

• ECs could be initiated from both the higher phenolic contained donor trees and the lower ones.

• However, the high phenolics contained lines produce less somatic embryos

  ▪ Chitin concentration in fungal inhibition test showed that high stilbene contained ECs showed stronger resistance against brown rot fungi

  ▪ Both UVC and fungi could induce STS gene expression. Responses of each line to induction are different.

  ▪ Stilbene accumulation in needles was correlated with the UVC radiation; UVC potential as screening method ??
Future works

- All the EC lines with high embryo production capacities have been cryopreserved for further research
- Over 2000 emblings grown in greenhouse for further testing
- Does the stilbene content measured from ECs correlate with fungal resistance of field-grown trees or wood from them?
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Thanks for your attention