Isolation of a TIR-NBS-like gene promoter from triploid white poplar and its characterization in transgenic tobacco plants

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Outline

- Background
- Our study
- Future work
Plant improvement for disease resistance

A major goal in plant science is the production of commercial plants with increased and durable resistance to a spectrum of diseases. In the past, two general approaches, including the conventional breeding and chemical treatment methods, have been sought. However, they are present to have several problems.

◆ Conventional breeding: Laborious, time-consuming (especially for the long-lived woody species, e.g. poplars)

◆ Chemical treatment: Expensive, hazardous to the environment
The plant genetic engineering

Genetic engineering has been used to introduce a set of valuable traits, such as pest resistance and herbicide tolerance, into a variety of commercial plants (e.g. Triploid white poplar).

The genetic engineering technology: offering an alternative avenue for the plant improvement with increased disease resistance.

The core genetic element: **transformation-cassette**

One of the pivotal problems for genetic engineering:
How to express the transgenes in the host plants?

- The high, constitutive promoters (e.g. CaMV 35S) ?
- The tissue/cell/organelle-specific promoters ?
- The inducible promoters ?

Overexpression of the defense component in transgenic plants may resulted in a set of problems, such as homology dependent gene silencing, unexpected disease symptoms, altered morphology and reduced size (dwarfish/stunted), especially for the perennial Poplar trees.
Our work

Focusing on the ‘Cloning and testing of the pathogen and/or defense signals inducible, tissue-specific promoters’, which may be promising for the Poplar genetic engineering in disease resistance.
The NBS-type resistance gene analogs (RGA) in triploid white poplar clone ‘L9’

Characterization of the *PtDrl02* gene

The RGA DQ324288

A TIR-NBS-like gene (*PtDrl02*)

Basal expression pattern of the *PtDrl02* gene in 18-month-old triploid white poplar
Inducible expression pattern of the *PtDrl02* gene

4-month-old seedlings

Time-course expression of the *PtDrl02* gene in response to defense-related signals
Isolation and computer analysis of the *PtDrl02* gene promoter

Genome walking

Sequencing of the primary PCR product
Function analysis of the PtDrl02 promoter

Tobacco transformation

<table>
<thead>
<tr>
<th>Transformant</th>
<th>Transgenic lines (n)</th>
<th>GUS staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-985</td>
<td>61</td>
<td>High</td>
</tr>
<tr>
<td>P-669</td>
<td>50</td>
<td>Medium</td>
</tr>
<tr>
<td>P-467</td>
<td>45</td>
<td>Low</td>
</tr>
<tr>
<td>P-244</td>
<td>50</td>
<td>Low</td>
</tr>
<tr>
<td>CaMV 35S</td>
<td>60</td>
<td>Strong</td>
</tr>
</tbody>
</table>
• Tissue-specific expression pattern of the *PtDrl02* promoter
Deletion analysis of the \textit{PtDrl02} promoter

P-985 \hspace{1cm} P-669 \hspace{1cm} P-467 \hspace{1cm} P-244

GUS activity (pmol 4-MU mg$^{-1}$ protein min$^{-1}$)

- P-985: GUS-NOS
- P-669: GUS-NOS
- P-467: GUS-NOS
- P-244: GUS-NOS

CaMV 35S \hspace{1cm} TATA \hspace{1cm} GUS-NOS

GUS activity (pmol 4-MU mg$^{-1}$ protein min$^{-1}$)

- CaMV 35S: GUS-NOS
• Activation of the *PtDrl02* promoter

![GUS activity graphs](image)

- [Control](#) vs. [Wound](#) with different treatments:
  - [MeJA](#)
  - [SA](#)

![GUS activity graphs](image)

- [Control](#) vs. [Treatment](#)
  - [Wound](#)
  - [MeJA](#)
  - [SA](#)
**Brief summary**

<table>
<thead>
<tr>
<th>Regulatory region</th>
<th>Inducers</th>
<th>Potential cis-elements</th>
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<tbody>
<tr>
<td>-985/-669</td>
<td>ABA</td>
<td>ABRE-motif</td>
</tr>
<tr>
<td>-669/-467</td>
<td>Wound, MeJA</td>
<td>W-box</td>
</tr>
<tr>
<td>-467/-244</td>
<td>SA, NaCl</td>
<td>GT-1-motif</td>
</tr>
<tr>
<td>-244/0</td>
<td>Wound, MeJA</td>
<td>W-box</td>
</tr>
</tbody>
</table>
• The PtDrl02 promoter activity is affected by its 5′ UTR

GUS staining of P-985/UTR
Predicted secondary structure of the *PtDrl02 5’ UTR*
Future work

- Analysis of the molecular interaction between *PtDrl02* promoter and its candidate transactivator *PtWRKY1*

- Examination of the orientation of *PtDrl02* promoter/cis-acting regulatory elements

- Testing of the *PtDrl02* promoter activity in the transgenic poplar (*P. tomentosa*) plants
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