Promoter tagging in banana (Musa spp.)
using the luciferase reporter gene -
development and applications

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Objective

- Isolate promoters from banana (*Musa* spp.) with constitutive, tissue specific, and cold stress responsive expression patterns
Content

I. The promoter tagging system

II. Luciferase expression patterns

III. Tagging of cold-responsive promoters

IV. Conclusions and perspectives
I. The promoter tagging system

promoter trap vector

Agrobacterium transformation

Screening for different parameters (abiotic and biotic stresses)
I. The promoter tagging system

**Luciferase reporter gene**

- Highly sensitive
- Non destructive screenings
- Short half-life of enzyme

\[
\text{Luciferin} + \text{ATP} + \text{O}_2 \xrightarrow{\text{LUC}} \text{Oxyluciferin} + \text{AMP} + \text{PPi} + \text{CO}_2 + \text{light (562 nm)}
\]

ultrasensitive digital CCD camera system
I. The promoter tagging system

High throughput screening for LUC activation

Months after *Agrobacterium* infection

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>...</th>
</tr>
</thead>
</table>

Medium

- ZZ
- RD1
- RD2
- Reg

Culture

- 400-600/sample
- 5,600-8,400/image
- 11,000-17,000/day

Screening

- ✓
- ✓
- ✓
- ✓
I. The promoter tagging system

Tagging constructs

pluc19

pETKUL2

pKCKUL1

662 bp

31 bp

pUbi-luc

pUbi-luc\(^+\)
### Effect of tagging constructs on activation frequency

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Tagging constructs</th>
<th>Total # of cell colonies screened</th>
<th>BLA frequency* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pLuc19</td>
<td>1,550</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>pET2</td>
<td>19,000</td>
<td>2.50</td>
</tr>
<tr>
<td>2</td>
<td>pET2</td>
<td>4,695</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>pET2</td>
<td>8,862</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>pKC1</td>
<td>33,390</td>
<td>2.03</td>
</tr>
</tbody>
</table>

* Baseline luciferase activity
II. Luciferase expression patterns

Cell colony stage

Live

pluc19 (luc) 0.06%

11,000 – 17,000 colonies/day

pETKUL2 (luc+) 2.5%
II. Luciferase expression patterns

*In vitro* plantlet stage

- **Live**
- **LUC**

**Constitutive expression**

**Root specific expression**
### III. Tagging of cold responsive promoters

<table>
<thead>
<tr>
<th>Temperature</th>
<th>2 months after transformation</th>
<th>4 months after transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIVE</td>
<td>26°C</td>
</tr>
<tr>
<td>18°C</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>16°C</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>12°C</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
</tr>
<tr>
<td>8°C</td>
<td><img src="image19.png" alt="Image" /></td>
<td><img src="image20.png" alt="Image" /></td>
</tr>
</tbody>
</table>

26°C Cold 2.5 h

Cold 2.5 h
Cold responsive luciferase activity (CRLA) at 8°C of transgenic cultures (~16,000) during regeneration

<table>
<thead>
<tr>
<th>Screening for CRLA</th>
<th>1st (Colony)</th>
<th>2nd (Cult)</th>
<th>3rd (Cult)</th>
<th>4th (Cult)</th>
<th>5th (Cult)</th>
<th>6th (Cult)</th>
<th>7th (Plantlet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lines showing CRLA</td>
<td>106</td>
<td>98</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>Percentage of lines showing CRLA</td>
<td>0.67%</td>
<td>0.62%</td>
<td>0.26%</td>
<td>0.26%</td>
<td>0.26%</td>
<td>0.18%</td>
<td>0.16%</td>
</tr>
</tbody>
</table>
III. Tagging of cold responsive promoters

Cell culture stage

pET2 lines

Live 26°C 8°C 26°C

Low luc expression

High luc expression

Relative Light Units

Time [hours]

26°C 8°C 26°C

17 17

28 28

Low luc expression

26°C

av1
III. Tagging of cold responsive promoters

*In vitro* plantlet stage

Temperature [°C]

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time [minutes]</th>
</tr>
</thead>
<tbody>
<tr>
<td>26°C</td>
<td>8°C</td>
</tr>
<tr>
<td>8°C</td>
<td></td>
</tr>
</tbody>
</table>

Relative Light Units

<table>
<thead>
<tr>
<th>Relative Light Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>80</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>120</td>
</tr>
<tr>
<td>140</td>
</tr>
<tr>
<td>160</td>
</tr>
</tbody>
</table>

Live

- pET2-34
- 26°C 20 min
- 8°C 3h40
III. Tagging of cold responsive promoters

Quantification of localized LUC expression in pseudostems of *in vitro* plantlets under cold stress

- **pET2-154**
  - Relative Light Units
  - Time [hours]

- **pET2-111**
  - Relative Light Units
  - Time [hours]

- **pET2-34**
  - Relative Light Units
  - Time [hours]

- **pET2-17**
  - Relative Light Units
  - Time [hours]
III. Tagging of cold responsive promoters

pET2-111

pET2-17
Conclusions

- Tagging constructs optimized
- Cold screening system developed
- Candidate lines obtained with enhanced and repressed CRLA patterns

Perspectives

- Confirmation of CRLA patterns in greenhouse plants
- Isolation of putative promoter sequences
- Bioinformatic analysis of promoter sequences