Regeneration and Genetic Transformation of Green Ash for Resistance to the Emerald Ash Borer

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Abstract

Objective

Develop a stable Agrobacterium-medium transformation system for genetically engineering green ash with resistance to the EAB.

Introduction

Green ash (F. pennsylvanica) is an important North American tree species. The wood is used for solid wood products, crates, boxes, and for specialty products such as tool handles, oars, and baseball bats because of the strength, hardness, shock resistance, and excellent bending qualities of the wood. Green ash is also very popular as a shade tree because of its good form, texture, and essential oil content which provides a sweet aroma when cut or crushed. But the emerald ash borer (EAB), an aggressive exotic beetle from Asia, has been found infesting green ash, white ash (F. americana), black ash (F. nigra), as well as several horticultural varieties of ash in Ohio, Indiana, Illinois, Michigan, Maryland and Ontario, and this devastation results in significant economic loss and environmental damage in these areas with EAB infestation (Dobesberger, 2002; Haack et al., 2002). To date there is no efficient means to completely eradicate the EAB. Genetic engineering is an efficient and feasible tool for introducing plants with resistance to pests compared to conventional breeding strategies. Adventitious shoot regeneration in tissue culture from plant organs is an important prerequisite for genetic modification via Agrobacterium-mediated transformation.

Results

1. Regeneration

Figure 2. Regeneration of plants from hypocotyls of green ash

Figure 3. Effect of BA and TDZ on shoot regeneration from hypocotyls

Table 1. Effect of Fraxinus pennsylvanica genotypes and auxin level on root formation of microshoots

<table>
<thead>
<tr>
<th>Auxin (µM)</th>
<th>IBA</th>
<th>Rooting (%)</th>
<th>Mean No. roots</th>
<th>Mean root length (cm)</th>
<th>Mean No. lateral roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.9</td>
<td>2.3</td>
<td>88.89</td>
<td>2.64±1.22</td>
<td>2.91±1.84</td>
<td>10.04±3.14</td>
</tr>
<tr>
<td>4.9</td>
<td>5.7</td>
<td>89.81±1.01</td>
<td>2.98±1.01</td>
<td>3.02±1.89</td>
<td>17.76±4.70</td>
</tr>
<tr>
<td>4.9</td>
<td>8.6</td>
<td>88.89±1.01</td>
<td>2.84±1.09</td>
<td>3.38±2.05</td>
<td>17.62±7.12</td>
</tr>
</tbody>
</table>

Table 2. Effect of Agrobacterium strains on transient expression of GUS

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of hypocotyls for transformation</th>
<th>No. of hypocotyls showing GUS transient expression</th>
<th>Transient expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBA4404</td>
<td>45</td>
<td>10</td>
<td>22.0</td>
</tr>
<tr>
<td>EHA105</td>
<td>48</td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>GV3101</td>
<td>50</td>
<td>10</td>
<td>33.3</td>
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Table 3. Shoot regeneration from hypocotyl on Kanamycin medium

<table>
<thead>
<tr>
<th>Kanamycin (µg/L)</th>
<th>Regeneration (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
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<tr>
<td>15</td>
<td>0</td>
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<tr>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

References


2. Primary result on transformation

Figure 4. GUS expression on the surface of hypocotyl

Figure 5. GUS expression at the end of hypocotyl

Figure 6. Elongation of kanamycin resistant shoots

Figure 7. Potted green ash

Figure 8. Acclimatization in the greenhouse

Materials and Methods

1. Regeneration

Adventitious shoot induction

Hypocotyl segments from 7-day-old embryos (Figure 2A) were cultured on Murashige and Skoog (MS) (1962) medium supplemented with 0.4, 4.9, 8.9, 13.3, or 22.2 µM 6-benzylaminopurine (BA) in combination with 0.5, 2.5, or 4.5 µM Thidiazuron (TDZ) for adventitious shoot induction.

Shoot elongation

Adventitious shoots (Figure 2B) were transferred to MS basal salt, B5 vitamins (MSB5) medium (Gamborg et al., 1968) supplemented with 10 µM BA plus 10 µM TDZ for shoot elongation (Figure 2C).

Rooting of micropropagated shoots and elongated adventitious shoots

Microshoots were cultured on woody plant medium (WPM) (Lloyd and McCown, 1988) supplemented with 4.9 µM Indole-3-butyric acid (IBA) in combination with 0.2, 2.9, 5.7, or 8.6 µM indole-3-acetic acid (IAA) for root formation. Microshoots were maintained for 10 days in the dark on rooting medium prior to transfer to a 16-hour photoperiod. Elongated adventitious shoots were cultured on WPM supplemented with 4.9 µM IBA plus 5.7 µM IAA to induce root formation (Figure 2D).

Accclimatization of rooted plants

Plants were transferred to a peat moss, vermiculite, and perlite mixture (1:1:1) to 6- to 8-inch plastic pots (Figure 2E). Plants were watered every two days. After 2 weeks of slow acclimatizing the plants in the lab, plants were transferred to the greenhouse. After another 2 weeks, plants were transferred to larger pots for further roots elongation (Figure 2F). Then plants were transferred to cold storage in-dorm for overwintering.

2. Genetic transformation

Kanamycin sensitivity

7-day-old hypocotyls were cultured on regeneration medium containing kanamycin at 0, 5, 10, 15, 20, or 30 mg/L for 6 weeks.

Transformation

The effect of three different Agrobacterium strains (LBA4404, EHA105, and GV3101) with binary vector (Figure 1) on transient expression of GUS was tested. Seven-day-old hypocotyls precultured for 2 days on regeneration medium (MS + 13.2 µM BA + 4.5 µM TDZ) were immersed in 20 ml bacterial solution (OD=0.6-1.0), vacuum infiltrated (20 inches of mercury) for 10 min plus 20 min on orbital shaker (100 rpm), blotted dry on sterile paper, transferred to regeneration medium, and plates incubated in the dark for 2 days. Explants were then washed three times (5 min/wash) using liquid MS medium and blotted dry. Explants were transferred to regeneration medium + Kan 15 mg/L + Tim 30 µg/L for regeneration. Some explants were used for GUS assay.

Conclusions

The best hypocotyl regeneration medium was MS supplemented with 13.3 µM BA plus 4.5 µM TDZ. The greatest percentage of hypocotyls regenerating was 76.3% and the mean number of adventitious shoots induced per hypocotyl was 1.7 ± 0.7. A high rooting percentage (73-90%) on WPM containing IBA plus IAA was achieved for three seedling genotypes and 93% rooting was achieved on WPM supplemented with 4.9 µM IBA plus 5.7 µM IAA for adventitious shoots. Rooted plants were successfully acclimated to the greenhouse and 100% survived overwintering in cold-storage. Kanamycin at 15 mg/L was suitable for use in selection medium.

Strain EHA105 was most suitable for transformation of hypocotyls.