PROPAGATION METHOD FOR SOFT CUTTINGS OF LIRIODENDRON

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Abstract

Genus Liriodendron (Magnoliaceae) comprises only two species, namely Liriodendron tulipifera L. and Liriodendron chinense (Hemsl.) Sarg. Stem cutting propagation experiment of the two species was carried out to speed up the asexual reproduction of Liriodendron. The best factor combinations for highest rooting rate were found out through orthogonal test. In the analysis of four factors including species, cuttings types, cutting medium and hormone concentration, the highest rooting rate came from the combination of L. tulipifera, stem cuttings with apical bud, peat soil and perlite (3 : 1) and ABT 380 ppm. In the analysis of three factors including seedling age, cutting medium and hormone concentration, the highest rooting rate came from the combination of cuttings from three years old Liriodendron, peat soil and perlite (3 : 1), and ABT 500 ppm. The key technique of cutting propagation of Liriodendron was created, which improved rooting rate of Liriodendron in the field planting significantly and reached a maximum to 60.9%. The propagation coefficient of two years old L. tulipifera cuttings was 5.05. Roots grew out of cuttings in the fourth week after planting, and the number of roots increased quickly during the fourth and fifth week. In the transplanting experiment, the survival rate of Liriodendron cuttings with roots reached up to 94.3%, and cuttings with callus had the survival rate of 45.9%. Lateral branches obtained from cuttings which had grown roots were transplanted and had the rooting rate of 88.6%, and the rate of cuttings with callus was 11.4%.

Introduction

Genus Liriodendron belonging to Magnoliaceae evolved from the origin type of oligocene with warm climate times. There are two species left in Liriodendron now. One is L. tulipifera, distributed in the east of USA (Little 1979). The other is L. chinense, located in the mountains at an elevation of 450 m to 1 800 m in southern China and northern Vietnam. The two species look quite similar from each other, and reproductive isolation does not exist between them. The hybrid of the two species was created in the 1960s’ in China. L. tulipifera (yellow poplar) is highly valuable as garden trees. The leaves look like Chinese T-shirt, and the color turns golden from green during autumn. The beautiful and fragrant flowers look like tulip with yellow perianth and delicate fragrance. The truck is high and straight, and the overall shape of the tree is neat and beautiful. L. tulipifera is also a valuable commercially wood and fiber species. It is regarded as a future renewable biomaterial because of quick growth with relatively high stress resistance (Liang et al. 2007, Jin et al. 2011). L. chinense is also ideal for landscaping because of its beautiful flowers and leaves. However, it is endangered in China, and this species was listed in the IUCN Red List of Endangered Plants in China, because of its low sexual reproductive efficiency (Fu and Jin 1992, Wang et al. 2012). However, the seed germination rate of Liriodendron is very low, which impacts on the rapid propagation and popularization of the species.

Softwood cutting is one kind of vegetative propagation, and has been used in many experiments. According to the previous multiple cutting test experience for many years, rooting time of cuttings of Liriodendron was found to be quite long, and root rot of the cuttings emerged frequently during
the period of cutting experiment. To provide information on the rapid asexual reproduction of *Liriodendron*, orthogonal test was conducted for finding out the optimal factor combination of cutting medium, hormone concentration, age of donor trees, and tree species, which have effect on improving rooting rate of cuttings. A key technique that could prevent root rot and accelerate rooting was developed in the present study. Propagation coefficient of 2 years old *Liriodendron* cuttings was calculated. In addition, rooting position and rooting time of *Liriodendron* cuttings were investigated, and transplanting survival rate of cuttings that had grown roots and cuttings which had grown callus was surveyed.

**Material and Methods**

Original trees were planted in Beijing and in the forestry farm of Jiangxi Province, in the South of China. Softwood cuttings were harvested from 2, 3 and 8 years old saplings of *L. tulipifera*, cuttings 1 year survival seedlings, and 3 years old saplings of *L. chinense*. Ten branches were cut from each tree, leaves were left on the branches and 2/3 piece of each leaf was cut off and 2-3 buds were kept on the branches.

To probe optimum treatment condition for stem cutting on *Liriodendron*, 2 orthogonal experiments were conducted out in 2013, the first one was on 13 July, 2013. L₈⁴ × 2³⁴ orthogonal experimental design was applied in this study (Table 1). Four factors namely varieties of donor trees, types of cuttings, cutting medium and hormone concentration were considered. The varieties of donor trees included *L. Tulipifera* (1) and *L. chinense* (2). The types of cuttings included stem cuttings with apical bud (1) and stem cuttings without apical bud (2); cutting medium included mixture of peat soil and perlite with proportion of three to one (1) and fresh pine sawdust (2); the concentration of ABT1 included 4 levels with 0 ppm (1), 380 ppm (2), 500 ppm (3), and 620 ppm (4). Eight blocks were used for planting cuttings, coded I to VIII, 80 cuttings were planted in each block, with a total number of 640 cuttings. The second orthogonal experiment was conducted on 20 July, 2013. L₈⁴ × 2²⁴ orthogonal experimental design was carried out in this study (Table 1). Stem cuttings with apical bud of *L. tulipifera* were used as experiment material. Three factors including ages of donor trees, cutting medium and hormone concentration, were considered. Ages of donor trees included two levels with 2 years old trees (1) and 3 years old trees (2); cutting medium included two levels with mixture of peat soil and perlite for the proportion of three to one (1) and fresh pine sawdust (2); the concentration of ABT1 included 4 levels with 0 ppm (1), 380 ppm (2), 500 ppm (3), and 620 ppm (4). Eight blocks were used for planting cuttings, coded I to VIII, 40 cuttings were planted in each block, with a total number of 320 cuttings. The orthogonal experiments were investigated and recorded in October, 2002. The data including percentage of rooting, percentage of callus tissue emergency, length of taproots, number of lateral roots, and rooting position (rooting from tissue culture or from lenticels) were recorded. Twenty samples were selected at random from each block, the 160 cuttings were used for analyzing for each orthogonal experiment. The factorial design analysis of variance provided by SAS10.0 software was used for these experiments. Percentage data, such as percentage of rooting, was analyzed after arcsine transformation.

The propagation difficulty of *Liriodendron* is presented as low rooting rate, root rot and slow speed of rooting. The techniques for preventing root rot and promoting rooting were conducted. Firstly, talcum powder and distilled water were blended into mushy. Secondly, carbendazin was joined into the mixture. ABT was a kind of rooting powder designed for hard rooting species by researchers of Chinese Academy of Forestry, and it was used as hormone in this experiment. A certain concentration of hormone was put into the mixture and then, all the ingredients were mixed by gently swirling until thoroughly combined. Finally, incisions, at the base of the cutting material,
were wrapped with the mixture, and then the bases of cuttings were inserted gently into cutting medium for 2 - 3 cm depth. The cutting materials should be managed and inserted into substance as quickly as possible to avoid inactivation. In the experiment, *L. tulipifera* was used as the donor trees for cuttings, concentration of ABT1 was 500 ppm, and the cutting medium was mixed with peat and perlite. In the first experiment 4505 samples were used and 9000 in the second one. All optical spray cutting pools provide a growth environment for samples. The growth condition was observed on autumn, 2013.

Table 1. The design for orthogonal experiments.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Concentration of ABT1</th>
<th>Types of cutting medium</th>
<th>Types of cuttings</th>
<th>Types of donor trees</th>
<th>No. of blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>First orthogonal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 levels</td>
</tr>
<tr>
<td>experiment design</td>
<td>L₈(4¹×2³)</td>
<td></td>
<td></td>
<td></td>
<td>2 levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 levels</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>2 levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 levels</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>2 levels</td>
</tr>
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<td>2 levels</td>
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<td></td>
<td></td>
<td></td>
<td>4 levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 levels</td>
</tr>
</tbody>
</table>

Table 1. The design for orthogonal experiments.

Number of cuttings provided by 2-years-old *L. tulipifera* saplings was observed. Four times biological repeats were taken in the experiment, and 30 samples were used in each observation. Lateral branches were obtained continuously from each sample during one growing season, and then were used for cottage propagation. The cuttings which had grown roots or callus were transplanted. Data were recorded, including rooting rate, transplanted survival rate of cuttings with roots, percentage of cuttings with callus, and transplanted survival rate of cuttings with callus. Propagation coefficient was calculated as follows: propagation coefficient = n × (r₁ × r₂ + r₃ × r₄).

In the above formula, n = a total number of lateral branches obtained continuously from each sample during one growing season for being cutting materials, r₁ = rooting rate of the cuttings, r₂ = transplanted survival rate of cuttings with roots, r₃ = percentage of cuttings with callus, and r₄ = transplanted survival rate of cuttings with callus.

The cutting materials were obtained from 2 years old *L. tulipifera*, including lateral branches with or without apical buds. Two types of cutting media were used. These were mixture with peat and perlite and fresh pine sawdust. The concentration of ABT was 500 ppm. Thirty six blocks were used for planting, and 20 cuttings were planted in each block, in a total of 720. When cutting propagation experiment was done after one week, observation was taken. Ten samples were selected in each week, and 3 times biological repeats were done. The observation continued for 8 weeks.
Results and Discussion

In the first orthogonal experiment, 80 cuttings were planted in each block, with a total number of 640 cuttings. The average number of cuttings with roots was 46.2, the average number of cuttings with callus was 15.9, and the average number of decayed cuttings which had from the incisions was 16.9 (Table 2) in each block. Block VI had the highest rooting rate which was 72.5\%, and the factor combination was \textit{L. tulipifera}, stem cuttings with apical bud, the media of peat soil and perlite (3 : 1) and ABT 380 ppm. Block II had the highest callus rate which was 38.8\%, and the factor combination was \textit{L. Chinense}, stem cuttings without apical bud, the media of pine sawdust and ABT 380 ppm. Block II had the highest percentage of cuttings with roots or callus which was 93.3\%, and the factor combination was \textit{L. Chinense}, stem cuttings with apical bud, media of peat soil and perlite (3 : 1) and ABT 500 ppm. Zhang and Gu (2003) reported that the survival rate of \textit{L. chinense} could be 21\% by using the combination of the mixture of rotten leaf soil and river sand as basic media and ABT 100 ppm which it was effective for cutting rooting. Hence, in comparison to the findings of Zhang and Gu’s (2003), the present combination was more effective.

Table 2. Data statistics on the first orthogonal experiment of \textit{Liriodendron}.

<table>
<thead>
<tr>
<th>Growth traits of cuttings</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cuttings</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Number of cuttings with roots</td>
<td>38</td>
<td>22</td>
<td>48</td>
<td>44</td>
<td>53</td>
<td>58</td>
<td>53</td>
<td>53</td>
<td>46.2</td>
</tr>
<tr>
<td>Number of cuttings with callus but without roots</td>
<td>26</td>
<td>31</td>
<td>13</td>
<td>8</td>
<td>4</td>
<td>9</td>
<td>22</td>
<td>13</td>
<td>15.9</td>
</tr>
<tr>
<td>Number of decayed cuttings</td>
<td>16</td>
<td>27</td>
<td>19</td>
<td>28</td>
<td>13</td>
<td>13</td>
<td>5</td>
<td>14</td>
<td>16.9</td>
</tr>
<tr>
<td>Percentage of cuttings with roots</td>
<td>0.475</td>
<td>0.275</td>
<td>0.600</td>
<td>0.550</td>
<td>0.663</td>
<td>0.725</td>
<td>0.663</td>
<td>0.662</td>
<td>0.577</td>
</tr>
<tr>
<td>Percentage of cuttings with callus</td>
<td>0.325</td>
<td>0.388</td>
<td>0.163</td>
<td>0.100</td>
<td>0.050</td>
<td>0.113</td>
<td>0.275</td>
<td>0.163</td>
<td>0.197</td>
</tr>
<tr>
<td>Percentage of cuttings with roots or callus</td>
<td>0.800</td>
<td>0.663</td>
<td>0.763</td>
<td>0.650</td>
<td>0.713</td>
<td>0.838</td>
<td>0.938</td>
<td>0.825</td>
<td>0.773</td>
</tr>
<tr>
<td>Percentage of cuttings with roots (arcsine transformation value)</td>
<td>0.495</td>
<td>0.279</td>
<td>0.644</td>
<td>0.582</td>
<td>0.724</td>
<td>0.811</td>
<td>0.724</td>
<td>0.724</td>
<td>0.623</td>
</tr>
<tr>
<td>Percentage of cuttings with callus (arcsine transformation value)</td>
<td>0.331</td>
<td>0.398</td>
<td>0.163</td>
<td>0.100</td>
<td>0.050</td>
<td>0.113</td>
<td>0.279</td>
<td>0.163</td>
<td>0.200</td>
</tr>
<tr>
<td>Percentage of cuttings with roots or callus (arcsine transformation value)</td>
<td>0.927</td>
<td>0.724</td>
<td>0.867</td>
<td>0.708</td>
<td>0.793</td>
<td>0.993</td>
<td>1.215</td>
<td>0.970</td>
<td>0.900</td>
</tr>
<tr>
<td>Number of cuttings used as measuring length of taproot</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Average length of taproots</td>
<td>8.53</td>
<td>4.40</td>
<td>9.64</td>
<td>8.58</td>
<td>9.65</td>
<td>8.69</td>
<td>8.15</td>
<td>7.75</td>
<td>8.17</td>
</tr>
<tr>
<td>Average number of total roots of cuttings</td>
<td>2.95</td>
<td>2.55</td>
<td>3.50</td>
<td>3.70</td>
<td>3.80</td>
<td>4.60</td>
<td>3.70</td>
<td>3.55</td>
<td>3.54</td>
</tr>
<tr>
<td>Average number of roots grown from callus</td>
<td>1.85</td>
<td>0.60</td>
<td>0.40</td>
<td>0.80</td>
<td>1.10</td>
<td>2.10</td>
<td>2.00</td>
<td>1.90</td>
<td>1.34</td>
</tr>
<tr>
<td>Average number of roots grown from lenticels</td>
<td>1.10</td>
<td>1.95</td>
<td>3.10</td>
<td>2.90</td>
<td>2.70</td>
<td>2.50</td>
<td>1.70</td>
<td>1.65</td>
<td>2.20</td>
</tr>
</tbody>
</table>
Twenty cuttings, which grew taproots, were selected randomly from each block to measure the length and number of cutting taproots. The results showed that, the average length of taproots for each block ranged from 4.4 to 9.6 cm, with a total average length of 8.2 cm, and the longest cutting taproot was 18 cm. The average number of taproots which grew from cuttings for each block ranged from 2.6 to 4.6, with a total average number of 3.5, and the highest number of cutting taproots in one block was 13. In this experiment, two positions where roots grew from cuttings were discovered from callus tissue or from lenticels. The average number of roots grew from callus was 1.3, and from lenticels was 2.3, which was higher than from callus.

Table 3. Data statistics on the second orthogonal experiment of *Liriodendron*.

<table>
<thead>
<tr>
<th>Growth traits of cuttings</th>
<th>Number of blocks</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cuttings</td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Number of cuttings with roots</td>
<td></td>
<td>25</td>
<td>12</td>
<td>21</td>
<td>15</td>
<td>11</td>
<td>27</td>
<td>32</td>
<td>24</td>
<td>20.9</td>
</tr>
<tr>
<td>Number of cuttings with callus but without roots</td>
<td></td>
<td>15</td>
<td>16</td>
<td>19</td>
<td>5</td>
<td>20</td>
<td>12</td>
<td>6</td>
<td>10</td>
<td>12.9</td>
</tr>
<tr>
<td>Number of decayed cuttings</td>
<td></td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>20</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>6.2</td>
</tr>
<tr>
<td>Percentage of cuttings with roots</td>
<td></td>
<td>0.625</td>
<td>0.300</td>
<td>0.525</td>
<td>0.375</td>
<td>0.275</td>
<td>0.675</td>
<td>0.800</td>
<td>0.600</td>
<td>0.522</td>
</tr>
<tr>
<td>Percentage of cuttings with callus</td>
<td></td>
<td>0.375</td>
<td>0.400</td>
<td>0.475</td>
<td>0.125</td>
<td>0.500</td>
<td>0.300</td>
<td>0.150</td>
<td>0.250</td>
<td>0.322</td>
</tr>
<tr>
<td>Percentage of cuttings with roots and callus</td>
<td></td>
<td>1.000</td>
<td>0.700</td>
<td>1.000</td>
<td>0.500</td>
<td>0.775</td>
<td>0.975</td>
<td>0.95</td>
<td>0.85</td>
<td>0.844</td>
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<tr>
<td>Percentage of cuttings with roots (arcsine transformation value)</td>
<td></td>
<td>0.675</td>
<td>0.305</td>
<td>0.553</td>
<td>0.384</td>
<td>0.279</td>
<td>0.741</td>
<td>0.927</td>
<td>0.644</td>
<td>0.563</td>
</tr>
<tr>
<td>Percentage of cuttings with callus (arcsine transformation value)</td>
<td></td>
<td>0.384</td>
<td>0.412</td>
<td>0.495</td>
<td>0.125</td>
<td>0.524</td>
<td>0.305</td>
<td>0.151</td>
<td>0.253</td>
<td>0.331</td>
</tr>
<tr>
<td>Percentage of cuttings with roots and callus (arcsine transformation value)</td>
<td></td>
<td>1.571</td>
<td>0.775</td>
<td>1.571</td>
<td>0.524</td>
<td>0.887</td>
<td>1.347</td>
<td>1.253</td>
<td>1.016</td>
<td>1.118</td>
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<tr>
<td>Number of cuttings used as measuring length of taproot</td>
<td></td>
<td>23</td>
<td>12</td>
<td>20</td>
<td>15</td>
<td>11</td>
<td>20</td>
<td>20</td>
<td>24</td>
<td>18.1</td>
</tr>
<tr>
<td>Average length of taproots</td>
<td></td>
<td>5.391</td>
<td>4.625</td>
<td>5.435</td>
<td>9.120</td>
<td>5.236</td>
<td>5.315</td>
<td>7.775</td>
<td>6.848</td>
<td>6.22</td>
</tr>
<tr>
<td>Average number of total roots of cuttings</td>
<td></td>
<td>3.30</td>
<td>2.08</td>
<td>2.70</td>
<td>4.47</td>
<td>2.73</td>
<td>3.15</td>
<td>4.85</td>
<td>3.67</td>
<td>3.37</td>
</tr>
<tr>
<td>Average number of roots grown from callus</td>
<td></td>
<td>1.73</td>
<td>2.08</td>
<td>0.95</td>
<td>4.27</td>
<td>2.73</td>
<td>0.95</td>
<td>4.3</td>
<td>1.22</td>
<td>2.28</td>
</tr>
<tr>
<td>Average number of roots grown from lenticels</td>
<td></td>
<td>1.57</td>
<td>0</td>
<td>1.75</td>
<td>0.20</td>
<td>0</td>
<td>2.20</td>
<td>0.55</td>
<td>2.45</td>
<td>1.09</td>
</tr>
</tbody>
</table>

In the second orthogonal experiment, 40 cuttings were planted in each block, with a total number of 320 cuttings. The average number of cuttings which grew roots was 20.9, the average number of cuttings which grew callus was 12.9, and the average number of cuttings which decayed...
from the incisions was 6.29 (Table 3). Block VII had the highest rooting rate which was 80.0%, and the factor combination included cuttings from 3-years old *L. tulipifera*, peat soil and perlite (3 : 1), and ABT 500 ppm. Block III had the highest callus rate of cuttings which was 47.5%, and the factor combination included cuttings from 2-years old *L. tulipifera*, pine sawdust, and ABT 500 ppm. Both block I and block I had the highest percentage of cuttings with roots or callus to 100%. The factor combination of block III included cuttings from 2-year-old *L. tulipifera*, peat soil: perlite (3 : 1), and ABT 0 ppm. The factor combination of block III included cuttings from 2-year-old *L. tulipifera*, pine sawdust and ABT 500 ppm. Twenty cuttings, which grew taproots, were selected randomly from each block to measure the length and number of cutting taproots. The results showed that, the average length of taproots for each block ranged from 4.6 to 9.1 cm, with a total average length of 6.2 cm, and the largest cutting taproot was 15.6 cm. The average number of taproots which grew from cuttings for each block ranged from 2.1 to 4.8, with a total average

<table>
<thead>
<tr>
<th>Dependent variable: x1</th>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>4</td>
<td>1 (0 ppm)</td>
<td>2 (380 ppm)</td>
<td>3 (500 ppm)</td>
<td>4 (620 ppm)</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>2</td>
<td>1 (peat)</td>
<td>2 (sawdust)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>2</td>
<td>1 (cuttings with apical buds)</td>
<td>2 (cuttings without apical buds)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>2</td>
<td>1 (<em>Liriodendron tulipifera</em>)</td>
<td>2 (<em>Liriodendron chinense</em>)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
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<td>a</td>
<td>3</td>
<td>0.02181868</td>
<td>0.00727289</td>
<td>0.20</td>
<td>0.8886</td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0.00732779</td>
<td>0.00732779</td>
<td>0.20</td>
<td>0.7313</td>
</tr>
<tr>
<td>c</td>
<td>1</td>
<td>0.12104907</td>
<td>0.12104907</td>
<td>3.33</td>
<td>0.3191</td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>0.01659789</td>
<td>0.01659789</td>
<td>0.46</td>
<td>0.6217</td>
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<th>F Value</th>
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<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>0.01650631</td>
<td>0.00550210</td>
<td>0.08</td>
<td>0.9619</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0.00028945</td>
<td>0.00028945</td>
<td>0.00</td>
<td>0.9589</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>1</td>
<td>0.01879357</td>
<td>0.01879357</td>
<td>0.27</td>
<td>0.6944</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>0.00039900</td>
<td>0.00039900</td>
<td>0.01</td>
<td>0.9518</td>
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<tr>
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<th>F Value</th>
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</tr>
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<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>0.05401230</td>
<td>0.01800410</td>
<td>0.63</td>
<td>0.7018</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0.02981316</td>
<td>0.02981316</td>
<td>1.05</td>
<td>0.4922</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>1</td>
<td>0.06940133</td>
<td>0.06940133</td>
<td>2.45</td>
<td>0.3622</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>0.01257636</td>
<td>0.01257636</td>
<td>0.44</td>
<td>0.6261</td>
<td></td>
</tr>
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<tr>
<th>Dependent Variable: x4</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>0.07843750</td>
<td>0.02614583</td>
<td>0.03</td>
<td>0.9917</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0.30031250</td>
<td>0.30031250</td>
<td>0.30</td>
<td>0.6829</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>1</td>
<td>1.08781250</td>
<td>1.08781250</td>
<td>1.07</td>
<td>0.4890</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>0.09031250</td>
<td>0.09031250</td>
<td>0.09</td>
<td>0.8155</td>
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<tr>
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<th>Mean Square</th>
<th>F Value</th>
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</tr>
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<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>8.03245937</td>
<td>2.67748646</td>
<td>0.36</td>
<td>0.8063</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0.77812813</td>
<td>0.77812813</td>
<td>0.10</td>
<td>0.8011</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>1</td>
<td>1.19737812</td>
<td>1.19737812</td>
<td>0.16</td>
<td>0.7574</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>1</td>
<td>1.82882813</td>
<td>1.82882813</td>
<td>0.25</td>
<td>0.7073</td>
<td></td>
</tr>
</tbody>
</table>
number of 3.4, and the highest number of cutting taproots in one block was 11. In this experiment, two positions where roots grew from cuttings were discovered mainly from callus tissue and few from lenticels. It was not similar to cuttings of *Hippophae rhamnoides* L. that was an integrated rooting type which mainly includes phloem rooting and minority of callus rooting (Li 2012). The average number of roots grown from callus was 2.3, and from lenticels was 1.1, which was lower than from callus.

Table 5. Analysis on the second orthogonal experiment.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>0.44640660</td>
<td>0.14880220</td>
<td>19.47</td>
<td>0.1647</td>
</tr>
<tr>
<td>b</td>
<td>1</td>
<td>0.02480408</td>
<td>0.02480408</td>
<td>3.25</td>
<td>0.3226</td>
</tr>
<tr>
<td>c</td>
<td>2</td>
<td>0.53638829</td>
<td>0.26819415</td>
<td>35.10</td>
<td>0.1185</td>
</tr>
</tbody>
</table>

Results of the first orthogonal experiment showed that, there was no significant difference between four factors including concentration of hormone (a), types of cutting medium (b), types of cuttings (c), and types of donor trees (d) in 5 indexes such as rooting rate (× 1), percentage of cuttings with callus (× 2), percentage of cuttings with roots or callus (× 3), average number of roots grown from cuttings (× 4), and average length of roots (× 5) (Table 4). Results of the second orthogonal experiment showed that, there was no significant difference between three factors including concentration of hormone (a), types of cutting medium (b), ages of donor trees (c) in 5 indexes such as percentage of cuttings with roots or callus (× 1), rooting rate (× 2), percentage of cuttings with callus (× 3), average number of roots grown from cuttings (× 4) (Table 5). However, there was significant difference between the three factors on the average length of roots (× 5) (α =
0.05). β test was conducted (β = 0.20) and indicated that two types of cutting media had the same effect on cutting growth, ages of donor trees and concentration of hormone had a few impacts on cuttings too, and impact from ages of donor trees was higher than from concentration of hormone. The cutting young-aged effect in *Liriodendron* was very obvious which was similar to other garden trees such as *Cedrus* and *Metasequoia glyptostyoides* (Yuan 2006).

In the first cutting experiment, 4505 cuttings were planted, 2742 samples grew roots (The rooting rate was 60.9%), 1205 samples grew callus (The percentage of cuttings with callus was 26.7%), and the number of samples where had roots or callus was 3947(87.9%). Nine thousand cuttings were planted in the second experiment which the samples were cut from donor trees when they stopped growing. The result showed that 2980 samples grew callus (The percentage of cuttings with callus was 33.1%) and didn’t grow roots, and it was supposed that the cutting time was too late to get root in this experiment. The rooting ability was also significantly affected in *Cinnamomum camphora* by the season of softwood cutting (Xiao et al. 2017).

**Table 6. Weekly Rooting rate of *Liriodendron tulipifera* in rooting experimental observation area.**

<table>
<thead>
<tr>
<th>Time Medium type</th>
<th>Week one - week three</th>
<th>Week four</th>
<th>Week five</th>
<th>Week six</th>
<th>Week seven</th>
<th>Week eight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat with perlite</td>
<td>0</td>
<td>4.4%</td>
<td>25.5%</td>
<td>46.8%</td>
<td>52.5%</td>
<td>54.2%</td>
</tr>
<tr>
<td>Pine sawdust</td>
<td>0</td>
<td>6.5%</td>
<td>26.9%</td>
<td>50.5%</td>
<td>53.2%</td>
<td>53.2%</td>
</tr>
<tr>
<td>Average value</td>
<td>0</td>
<td>5.45%</td>
<td>26.20%</td>
<td>48.65%</td>
<td>52.85%</td>
<td>53.70%</td>
</tr>
</tbody>
</table>

Propagation coefficient is very important for excellent varieties application in production. The propagation coefficient of 2-year-old *L. tulipifera* was investigated in this experiment to acquire the cutting propagation speed of *L. tulipifera*. The result showed that, each sample could provide 6.7 effective lateral branches for cutting materials in one growing season in average. In this survey, rooting rate of the cuttings was 66.3% and transplanted survival rate of cuttings with roots was 94.7%; callus rate of cuttings was 27.5%, and transplanted survival rate of callus cuttings was 45.9%. According to the computational formula, the propagation coefficient was 5.05 (= 6.7× (66.3 × 94.7% + 27.5 × 45.9%)) which was higher than that of *Stachys sieboldii* and its rooting rate of the cutting seedlings in autumn was only about 10 % and the propagation coefficient was 2.5 (Li et al. 2008).

According to the record, types of cutting medium had no significant effect on rooting time and rooting quantity of cuttings in this experiment. Callus began to emerge from the second week after planted. Top of roots began to emerge from the third week, and real roots with the length of 2 -5 mm grew in the fourth week. The number of roots increased quickly during the fifth and sixth week, and the growth rate of rooting reduced significantly in the seventh and eighth weeks (Table 6). It was considered that, the roots of cuttings of *L. tulipifera* emerged from the fourth week after planted, and the rooting rate increased quickly during the fifth and sixth week (Fig. 1).

In this experiment, 764 cuttings with roots survived with survival rate of 94.3%, and 62 cuttings with callus survived with survival rate of 45.9% (Fig. 2) which showed nearly 50% of the samples with callus but lack of roots succeeded to live in the transplanting, which indicated a strong vitality of *L. tulipifera*.

There are differences on the growth condition of cuttings of *Liriodendron* in cutting propagation experiment between combinations of treating factors. Specifically, the best hormone concentration of ABT was 500 ppm, cuttings with apical buds were better than cuttings without apical buds. The survival rate of cuttings from one-year-old saplings is higher than cuttings from...
8-year-old trees. There is no difference between *L. tulipifera* and *L. chinense* on the growth condition in the experiment. The optimal combination of factors obtained from this experiment would be verified in the further field trial to facilitate the future promotion and improve the asexual reproduction rate of *L. chinense*. It would not only generate considerable economic benefits but also play an important role in protecting *L. chinense* from extinction.

In the experiment, the highest survival rate of cuttings including cuttings with roots or callus was 100%, and the average was nearly 90%. It proved further that this technique could be used in large-scale field cuttings and production application. At present, the key technique has been used by many research institutions and enterprises for seedling, for instance, Beijing Ming tombs forest farm and Beijing Zhong Lin Land Breeding Company, where the survival rate of cuttings has been increased significantly.
Acknowledgments

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References


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