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In vitro propagation of oil-bearing rose (*Rosa damascena* Mill.)

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**Abstract.** The purpose of this research is to develop effective protocol for clonal micro propagation of the oil-bearing rose (*Rosa damascena* Mill.). Explants used in the experiment are 1.0 to 1.5 cm long nodal segments from specially cultivated mother plants. These explants were subjected to a combined method of sterilization with 0.2% solution of HgCl₂, for 3 minutes followed by 0.50% solution of NaClO for 20 minutes and 0.25% solution of NaClO for 30 minutes. The best results of multiplication were obtained in basic MS medium with added BAP quantity of 0.5 to 3.0 mg/L. After being separated from the mother plants the young shoots are placed directly in compost mixture for rooting and adaptation. Thus the process of multiplication is shortened, avoiding the period of in vitro rooting.

**Keywords:** oil-bearing rose, micropropagation, protocol

**Abbreviations:** MS - Murashige & Skoog medium, IBA - Indole-3-butyric acid, BAP - 6-Benzylaminopurine, TDZ - Thidiazuron

**Introduction**

The oil-bearing rose has been the most symbolic industrial crop of Bulgaria. First in the world is the renowned Kazanlak oil-bearing rose (*Rosa damascena* Mill.*f. trigintipetala* Dieck). It has been cultivated for the production of rose oil, rose concrete and rose absolute which are widely used in cosmetics, pharmaceutics and the food industry (Kovacheva and Nedkov, 2007). Presently, oil-bearing rose planting material is mainly obtained from the rooting of sprout cuttings in a cultivation facility, a technology developed by the Institute of Roses, Essential and Medical Cultures in Kazanlak in 1986 (Atanasova and Nedkov, 2004). The first announcement of oil-bearing rose in vitro propagation in Bulgaria has been made by Kornova et al. (2001). Thorpe and Harry (1997) in their own research proved that in vitro techniques were the fastest, the most effective and the easiest way of plant propagation and played a major role in the production of biologically clean and quality planting material.

In research made by Mamaghani et al. (2010) the use of different kinds of hormones combination and concentration by the plants proliferation process was tested. In this research three genotypes of *Rosa damascena* Mill. were developed. They found out that best results are achieved on MS medium with addition of 5mg/L BAP+0.1mg/L TDZ. In study of Ginova and Konadakova (2014) the advantages of in vitro cultivation with bioreactors are shown. Object of this research was the oil-bearing rose. With all the experience until now, this research has been conducted in order to produce a working protocol for clonal micropropagation of the oil-bearing rose. This technology should provide a more effective system for producing plant material in industrial conditions, keeping the quality of plants, finishing the process in shorter production time and using less space for initial growing. Regarding the needs of the new oil-bearing rose plantations the research will be an answer.

**Material and methods**

In this research „Population No. 5“ of *Rosa damascene* Mill. has been given a laboratory number R3, and the „Eleyna“ cultivar - a laboratory number R3-4, respectively. The plant material has been obtained from elite field crop trials of the Institute of Roses, Essential and Medical Cultures in the town of Kazanlak. The test was conducted in a laboratory for in vitro propagation of INDUSTRIAL PLANTS, LLC, town of Kazanlak and involved three main stages: culture initiation, micropropagation and rooting of the obtained plants. 8-10 cm long nodal segments from stock plants cultivated to this purpose were used as initial explants. After disinfection the plant material was cut into segments 1.0-1.5 cm long, with 1-2 axillary buds just before placing them into a growth medium, each of them in closed glass dishes (Senapati and Rout, 2008). Three types of disinfection were researched, the explants being preliminarily treated with 70% ethylic alcohol on a magnetic stirrer (Table 1.)

The first type involves treatment in 0.2% HgCl₂ solution for 5 minutes. Rinsing in sterile water three times for 5 minutes in a laminar air flow chamber, immediately before placing in growth medium (Ginova and Konadakova, 2013).

The second type involves treatment in 0.5% NaClO solution for 20 minutes and in 0.25% NaClO solution for 30 minutes, constantly stirred on a magnetic stirrer. Rinsing in sterile distilled water three times for 5 minutes.

The third type of disinfection runs in two stages. First stage – 0.2% HgCl₂ solution for 3 minutes and rinsing in sterile distilled water for 2 minutes. Second stage – disinfection in 0.5% NaClO solution for 20 minutes and rinsing in sterile distilled water for 2-3 minutes. The sterilization process continues in 0.25% NaClO solution for 30 minutes and rinsing in sterile distilled water.

A total of 40 explants of each genotype were set up for each type of disinfection. One explant was placed per jar and cultivated in...
premises with controlled conditions at 21°C, illuminance 2000 lux, cool white, period light/dark - 12/10, for 20 days. The induction and proliferation growth medium was MS (Murashige and Skoog, 1962), hormones free. The medium was hardened with Agar 6 g/L., and pH was fixed at 6.0 before sterilization which was conducted at 121°C under 1.1 atm., for 20 minutes. The disinfected live explants, 20 days after culture induction were transferred in multiplication media. The media were alkaline MS with different combinations of 8 types of growth regulators added, specified in Table 2, 4 pcs. in one glass jar. The viable explants were transferred onto a fresh medium every 4 weeks. The root formation process for both genotypes passed through 2 types specified in Table 3. First type – one part of the sprouts was placed in a rooting medium. The medium was MS with different auxins added and MS unchanged and hormones free (Canli and Kazaz, 2009). Under such

Table 1. Impact of disinfection over explants

<table>
<thead>
<tr>
<th>Disinfection variant</th>
<th>Genotype</th>
<th>Number of explants</th>
<th>Alive pure explants</th>
<th>Died explants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>number</td>
<td>%</td>
</tr>
<tr>
<td>1. 0.2% solution of HgCl₂ for 5 min</td>
<td>R3</td>
<td>40</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>R3-4</td>
<td>40</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Total for variant 1</td>
<td></td>
<td>80</td>
<td>17</td>
<td>21.25</td>
</tr>
<tr>
<td>2. 0.5% solution for NaClO for 20 min and 0.25% for 30 min</td>
<td>R3</td>
<td>40</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>R3-4</td>
<td>40</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Total for variant 2</td>
<td></td>
<td>80</td>
<td>13</td>
<td>16.25</td>
</tr>
<tr>
<td>3. 0.2% solution of HgCl₂ for 3 min + 0.5% solution for NaClO for 20 min and 0.25% for 30 min</td>
<td>R3</td>
<td>40</td>
<td>29</td>
<td>72.5</td>
</tr>
<tr>
<td></td>
<td>R3-4</td>
<td>40</td>
<td>32</td>
<td>80</td>
</tr>
<tr>
<td>Total for variant 3</td>
<td></td>
<td>80</td>
<td>61</td>
<td>76.25</td>
</tr>
</tbody>
</table>

* death infected, # death from the disinfectant

Table 2. Influence testing of different kind and concentration of growth hormones over the multiplication process

<table>
<thead>
<tr>
<th>Medium variant: R3</th>
<th>Amount of used explants</th>
<th>Shoots per explant</th>
<th>Shoots total</th>
<th>Total multiplied explants, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Explants with presented number of shoots</td>
<td>0 1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>1. MS+ 0.5 BAP mg/L</td>
<td>20</td>
<td>1 1 3 2 5 8</td>
<td>73</td>
<td>95</td>
</tr>
<tr>
<td>2. MS+ 3.0 BAP mg/L</td>
<td>20</td>
<td>0 0 2 6 4 5</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>3. MS+ 5.0 BAP mg/L</td>
<td>20</td>
<td>0 6 3 6 2 3</td>
<td>53</td>
<td>100</td>
</tr>
<tr>
<td>4. MS+ 0.5 BAP+1.5 kinetin mg/L</td>
<td>20</td>
<td>3 1 7 6 1 2</td>
<td>47</td>
<td>85</td>
</tr>
<tr>
<td>5. MS+ 0.5 BAP+1.5 zeatin mg/L</td>
<td>20</td>
<td>3 9 7 1 0 0</td>
<td>26</td>
<td>85</td>
</tr>
<tr>
<td>6. MS+ 0.5 BAP+1.5 TDZ mg/L</td>
<td>20</td>
<td>5 9 4 0 1 1</td>
<td>26</td>
<td>75</td>
</tr>
<tr>
<td>7. MS+ 0.5 BAP+0.5 CPPU mg/L</td>
<td>20</td>
<td>9 6 3 1 1 0</td>
<td>19</td>
<td>55</td>
</tr>
<tr>
<td>8. MS mod+ 0.5 BAP+OX</td>
<td>20</td>
<td>9 8 1 2 0 0</td>
<td>16</td>
<td>55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medium variant: R3-4</th>
<th>Amount of used explants</th>
<th>Shoots per explant</th>
<th>Shoots total</th>
<th>Total multiplied explants, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Explants with presented number of shoots</td>
<td>0 1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>1. MS+ 0.5 BAP mg/L</td>
<td>20</td>
<td>0 1 3 7 6 3</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>2. MS+ 3.0 BAP mg/L</td>
<td>20</td>
<td>1 2 3 9 2 3</td>
<td>58</td>
<td>95</td>
</tr>
<tr>
<td>3. MS+ 5.0 BAP mg/L</td>
<td>20</td>
<td>2 3 5 6 4 0</td>
<td>47</td>
<td>90</td>
</tr>
<tr>
<td>4. MS+ 0.5 BAP+1.5 kinetin mg/L</td>
<td>20</td>
<td>3 8 6 1 1 1</td>
<td>32</td>
<td>85</td>
</tr>
<tr>
<td>5. MS+ 0.5 BAP+1.5 zeatin mg/L</td>
<td>20</td>
<td>4 6 9 0 1 0</td>
<td>28</td>
<td>80</td>
</tr>
<tr>
<td>6. MS+ 0.5 BAP+1.5 TDZ mg/L</td>
<td>20</td>
<td>3 8 5 3 0 1</td>
<td>32</td>
<td>85</td>
</tr>
<tr>
<td>7. MS+ 0.5 BAP+0.5 CPPU mg/L</td>
<td>20</td>
<td>9 7 3 0 1 0</td>
<td>17</td>
<td>55</td>
</tr>
<tr>
<td>8. MS mod+ 0.5 BAP+OX</td>
<td>20</td>
<td>11 4 2 2 1 0</td>
<td>18</td>
<td>45</td>
</tr>
</tbody>
</table>

*ox (vitamin C and citric acid 50 mg/L)
conditions, the root formation process was analyzed within 4 weeks. Second type – the other part of young sprouts were placed in compost mixture 1:1 /perlite:peat/ for direct rooting, without passing through a rooting medium (Pittet and Moncousin, 1982). Before placing them in compost mixture they had to be soaked in IBA-0.1 mg/L solution for 1 minute while gently stirring the recipient in which they were treated.

After rooting young plants were transferred in a closed greenhouse under conditions of high humidity 80-90% and at 30-32˚C for 20 days. After a period of annealing during which the temperature and humidity were gradually lowered, the greenhouse was finally opened.

Results and discussion

Suitable explants were obtained from the secondary growth of plants during the period end June - beginning of July, after flowering. Three types of disinfection were researched. A detailed post-analysis determined that the most suitable combination was a 0.2% HgCl₂ solution for 3 minutes, subsequently treated in a 0.5% NaClO₂ solution for 20 minutes and in a 0.25% NaClO solution for 30 minutes. In this case we obtained the maximum number of viable pure explants. In the 0.1% HgCl₂ solution for 5 minutes type, most of the explants did not survive the sterilization process and died. When treated in a 0.5% NaClO solution for 20 minutes, followed by a 0.25% NaClO solution for 30 minutes, most of the explants were found infected. These results are valid for both genotypes (Table 1).

During the multiplication period studied growth regulators were used in different concentrations. The results from the analysis showed that the highest percentage of multiplied explants, as well as the highest number of sprouts per explant, were obtained in a MS medium not changed with a BAP additive, in quantities from 0.5 to 3.0 mg/L. In the course of work it became clear that if BAP quantity was increased or combined with another hormone, the number of sprouts and the total percentage of multiplications diminished. This was even more valid for R3 (Table 2). The relation between the medium variant and the multiplication power is presented in Figure 1.

The rooting process ran equally well on a root formation medium, as well as through direct rooting in compost mixture. When

![Figure 1. Relation between the medium and degree of multiplication](image-url)

Table 3. Rooting process with or without using medium for rooting

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rooting variant</th>
<th>Amount of used explants</th>
<th>Amount of rooted explant</th>
<th>% of rooting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.MS- without hormones</td>
<td>40</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>Variant I</td>
<td>2.MS+0.1 mg/L IBA</td>
<td>40</td>
<td>36</td>
<td>90</td>
</tr>
<tr>
<td>Using medium for rooting</td>
<td>3. MS+0.1 mg/L IAA</td>
<td>40</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>4. MS+0.1 mg/L NAA</td>
<td>40</td>
<td>31</td>
<td>77.5</td>
</tr>
<tr>
<td>Variant II</td>
<td>Soil mix</td>
<td>40</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Direct rooting</td>
<td>Total for the genotype</td>
<td>200</td>
<td>147</td>
<td>73.5</td>
</tr>
<tr>
<td>Variant I</td>
<td>1.MS- without hormones</td>
<td>40</td>
<td>32</td>
<td>80</td>
</tr>
<tr>
<td>Using medium for rooting</td>
<td>2.MS+0.1 mg/L IBA</td>
<td>40</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3. MS+0.1 mg/L IAA</td>
<td>40</td>
<td>31</td>
<td>77.5</td>
</tr>
<tr>
<td></td>
<td>4. MS+0.1 mg/L NAA</td>
<td>40</td>
<td>28</td>
<td>70</td>
</tr>
<tr>
<td>Variant II</td>
<td>Soil mix</td>
<td>40</td>
<td>38</td>
<td>95</td>
</tr>
<tr>
<td>Direct rooting</td>
<td>Total for the genotype</td>
<td>200</td>
<td>169</td>
<td>84.5</td>
</tr>
</tbody>
</table>

Conclusion

The analysis of the results from the conducted research answers several major questions. The initial material should be from elite stock plants grown under controlled conditions. Explants would be nodal segments 1-1.5 cm long with 1 to 2 axillary buds. The most appropriate protocol for sterilization was recognized to be a combination of 0.2% HgCl₂ solution for 3 minutes, followed by a treatment in 0.5% NaClO solution for 20 minutes and in 0.25%
NaClO solution for 30 minutes. During the multiplication period the biggest number of sprouts and the highest percentage of multiplied explants were obtained in MS medium with added BAP in concentration from 0.5 to 3.0 mg/L. The process of root formation did not require in vitro conditions and a rooting medium. Young sprouts could be placed directly in compost mixture and rooted directly under in vivo conditions.

References

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**Conclusion:** The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn’t be numbered and no new paragraphs be used. Contributions are the core of conclusions.

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**References**


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