Clonal Micropropagation, Rhizogenesis and Adaptive Capacity of Certain Rose (Rosa L.) Variety Explants

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Abstract
This article presents research on the inclusion of the biotechnological link in rose breeding. Roses are heterozygous plants and give splitting when propagated by seed. Therefore, clonal micropropagation has been included in the research to reproduce and preserve valuable genotypes and new starting material. The aim of this study was to select the type of auxin and its optimal concentrations for better Rhizogenesis of micro-sprouts of selected rose cultivars, and to select the suitable substrate for more effective adaptation of regenerant plants. For the introduction of explants into culture, cultivation and preparation of nutrient media, the traditional methods adopted in tissue culture work were applied. For rooting of well-formed micro-sprouts, liquid nutrient medium with a halved content of macro- and microsalts and sucrose was used. In addition, different concentrations of 3-indolyl butyric acid and β-indolyl-3-acetic acid were added to the medium. Based on the findings of the study, it is recommended to use indolyl butyric acid at concentrations of 0.5 mg/l and 1.0 mg/l for clonal micropropagation of roses, since at these concentrations, the rooting frequency indices by varieties were the highest. When adapting the plant material, it is better to use a substrate consisting of peat, soddy soil and perlite in a ratio of 1:1:1. The best results in the studies were noted in the varieties Alan Titchmarsh, Coral Surprise and Tchaikovski.

Keywords
Rosa L.; Clonal micropropagation; Rhizogenesis; Adaptation
Introduction

The genus *Rosa* (*Rosa* L.) belongs to the family *Rosaceae* Juss. and includes about 200 species (Ku and Robertson, 2003; Wissemann, 2003; Raymond et al., 2018). However, some scientists note that this number of species reaches up to 400 species (Khrzhanovsky, 1958; Saakov, 1958). Rose, since ancient times, is one of the main ornamental plants. The genus *Rosa* L. is widely distributed throughout the world. Scientists believe that the primary centers of origin of roses are North and South Asia (China, India) and Near East Asia (Transcaucasia, Iran), from where they were introduced to Europe (Greece and Rome) by missionaries in the 14th century (Saakov and Rieks, 1973; Klimenko and Klimenko, 1974; Rubtsova, 2009; Bhattacharjee, 2010). The first mentions of roses in the territory of Ukraine date back to the 17th century. The rose became widespread in the country since the 19th century (Rubtsova, 2009).

Nowadays, roses are economically important ornamental plants in the world. There are more than 37,000 commercial rose varieties of different groups (The American Rose Society, 2021). Most roses are heterozygous (Gudin, 2000) and give significant splitting when propagated by seed. Therefore, they are usually propagated vegetatively to maintain the grading factors (Khosravi et al., 2007; Rubtsova, Chyzhankova and Boiko, 2015). Vegetative propagation is a complicated, long and exhausting process, and according to some scientists, this process is not good for rose propagation (Khosravi et al., 2007). An alternative to vegetative propagation is the use of biotechnological techniques. These methods are used for accelerated propagation (it is possible to clone up to 400,000 plants per year from one rose plant), to preserve valuable plants and to solve breeding problems (Claude, 1985). In the world practice, the method of cell, tissue and organ culture is most commonly used, the method of clonal micropropagation is widely used for accelerated reproduction of new starting material and existing one for reproduction and conservation of valuable genotypes (Altman, 2000; Kushnir and Sarnatskaya, 2005; Jain et al., 2006).

Clonal micropropagation consists of a number of consecutive stages, which can be divided into the main four ones: 1) selection of a donor plant, isolation of explants and obtaining sterile viable culture; 2) own clonal micropropagation; 3) rooting of micro-sprouts in *in vitro* conditions; 4) transfer of regenerated plants from *in vitro* to *ex vitro* conditions and subsequent adaptation (Kushnir and Sarnatskaya, 2005; Khosravi et al., 2007; Canli and Kazaz, 2009).

Scientists have formulated that nutrient media should provide all the necessary elements and nutrients needed for plant growth *in vitro*. Hence, the selection or development of a nutrient (culture) medium is an important step in any tissue culture project. In clonal micropropagation of plants, the commonly used isolated tissue culture media are used: (Murashige and Skoog, 1962; Gamborg and Eveleigh, 1968), and other nutrient media with different modifications of the hormonal composition (Melnichuk, Novak and Kunah, 2003; Krasilnikova, Avksentyeva and Zhmurko, 2007; Melnichuk and Klyachenko, 2014). Currently, most studies on clonal micropropagation of commercial varieties are aimed at optimizing the composition of nutrient medium, the ratio of phytohormones and their concentration (Musienko and Paniuta, 2001). When carrying out clonal micropropagation, it is necessary to take into account the factors that affect this process. Hence, at the initial stage the season of material isolation, plant genotype and its age etc. have influence (Kumar and Reddy, 2011). Scientists have proved that organs and tissues isolated in the period of plant active vegetation are better adapted to the *in vitro* conditions compared to the material isolated in the period of deep dormancy (Pilunskaya and Bugara, 2001; Yegorova and Stavzeva 2016).

In the literature, scientists have proposed a number of sterilization schemes to obtain a high ratio of sterile viable explants. However, scientists using known techniques continue to develop new ones, since the same organ in different genotypes requires specific sterilization conditions (Kalinin, 1992; Melnichuk, Novak and Kunah, 2003; Kushnir and Sarnatskaya, 2005; Sidenko and Mitrofanova, 2011). Hormonal factor and nutrient medium composition are factors influencing the clonal micropropagation process. In addition, the
acidity of the nutrient medium (optimal pH reaction is 5.6-6.5), temperature, relative humidity and room lightning affect the process (Kushnir and Sarnatskaya, 2005).

Some scientists note that during in vitro rooting of micro-sprouts the medium must contain a reduced amount of salts than the medium on which the propagation was carried out (Aydinli and Tutas, 2003; Khosravi et al., 2007; Bitics et al., 2008). Rooting of micro-sprouts is influenced by genetic characteristics of the starting material, type of auxin and its concentration. Some researchers note that the in vitro rooting process is influenced by the ratio of growth regulators at the stage of micropropagation (Kondratenko and Mitrofanova, 2002).

When going through the fourth in vitro stage, it is necessary to pay attention to the condition of the regenerant plant and physical factors (Kirichenko, Kuzmina and Kataeva, 1991; Basim and Basim, 2003). Some scientists consider this period as the most critical and stressful for regenerant plants (Upadyshev and Vysockij, 1992). The process of plant-regenerants’ adaptation to soil conditions is the most expensive and time-consuming operation. Often, after transplanting the plants into the soil, there is a stoppage in growth, leaf fall and death of plants. These phenomena are associated, first of all, with the fact that the activity of the stomata of regenerated plants is disturbed, resulting in the loss of a large amount of water (Dedyukhina, Konstantinova and Baranova, 2011).

The objective of this research is to select the optimal conditions for the process of clonal micropropagation at all stages. However, the main objective of is study to select the type of auxin and its optimum concentration for better rooting of micro-sprouts, and to select the best substrate for more effective adaptation and to identify the varieties best suited for clonal micropropagation.

**Material and Methods**

The research was conducted in the educational-research-production laboratory of biotechnology of Uman National University of Horticulture. The valuable varieties with decorative properties and different originators were used, such as: Reimer Kordes (Germany) – “Gebruder Grimm” (2002), “Lavaglut” (1978), “Friesia” (1973) – belong to the genotypes of Floribunda groups; Meilland International (France) – “Tchaikovski” (2000) – belong to the genotypes of Floribunda group; Z.K. Klymenko (Ukraine) – “Coral Surprise” (1966) - belongs to the genotypes of Grandiflora group.; David Austin (UK) – “Alan Titchmarsh” (2000) - belongs to the genotypes of the group Shrab, also belongs to the group of varieties – English roses (Table 1).

<table>
<thead>
<tr>
<th>№</th>
<th>Variety name</th>
<th>Plant height, cm</th>
<th>Flower shape</th>
<th>Flower color</th>
<th>Number of petals, pcs.</th>
<th>Flower diameter, cm</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gebruder Grimm</td>
<td>70-90</td>
<td>Irregular-rounded</td>
<td>Orange and yellow</td>
<td>26-40</td>
<td>9.4-10.7</td>
<td>weak</td>
</tr>
<tr>
<td>2.</td>
<td>Lavaglut</td>
<td>60-75</td>
<td>Rouded</td>
<td>Dark red</td>
<td>26-40</td>
<td>4.5-6.5</td>
<td>moderate</td>
</tr>
<tr>
<td>3.</td>
<td>Friesia</td>
<td>70-80</td>
<td>Irregular rounded</td>
<td>-</td>
<td>30-35</td>
<td>7.5-8.5</td>
<td>strong</td>
</tr>
<tr>
<td>4.</td>
<td>Tchaikovski</td>
<td>60-80</td>
<td>Rounded</td>
<td>White and yellow</td>
<td>90-110</td>
<td>8.7-10.0</td>
<td>strong</td>
</tr>
<tr>
<td>5.</td>
<td>Coral Surprise</td>
<td>60-80</td>
<td>Irregular rounded</td>
<td>-</td>
<td>25-30</td>
<td>10.2-11.8</td>
<td>moderate</td>
</tr>
<tr>
<td>6.</td>
<td>Alan Titchmarsh</td>
<td>100-120</td>
<td>Rounded</td>
<td>Light blue and pink</td>
<td>90-100</td>
<td>11.3-13.7</td>
<td>moderate</td>
</tr>
</tbody>
</table>
The axillary buds (explants) with 0.5 – 0.8 cm size were selected from plants typical in phenotype and not affected by diseases and pests. In general, explants were selected during the growing season of roses (at the stages of active vegetation and dormancy). First period - at the beginning of the growing season (March); second period - during active growth (July); third period - at the end of the growing season of the varieties studied (October). The studies included three replicates. Cultivation was carried out according to generally accepted methods, in a light room with standard indices of illumination (1500-3000 Lux), temperature (25°C), relative humidity (65-70%) and a photoperiod of 16 hours as suggested by earlier workers (Melnichuk, Novak and Kunah, 2003; Kushnir and Sarnatskaya, 2005; Melnichuk and Klyachenko 2014).

To optimize the sterilization of the axillary buds it was performed in several stages using three sterilizing agents with different exposure periods. Exposition of sterilization with 70% ethyl alcohol was for 1-3 minutes, with mercury dichloride (HgCl₂ – 0.1%) for 3-7 minutes, and with sodium hypochlorite (NaClO - 1: 3) for 10-20 minutes. To root the well-formed micro-sprouts, a nutrient medium with a halved content of macro- and micro-salts and sucrose was used. In addition, different concentrations of 3-indolebutyric acid (IBA) and β-indolyle-3-acetic acid (IAA) were added to the medium (Table 2). Liquid culture medium was used for rooting, based on the research of scientists who indicated a higher percentage of rooting on liquid nutrient medium than on medium containing agar-agar (Kornova, Mihailova and Stefanova, 2001).

### Table 2: Modifications of Murasige and Skuga nutrient medium to initiate rose Rhizogenesis *in vitro*

<table>
<thead>
<tr>
<th>Variant</th>
<th>Modification MS</th>
<th>The composition of the nutrient medium, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS (St)</td>
<td>1/2 MS + without phytohormones</td>
</tr>
<tr>
<td>2</td>
<td>MS7</td>
<td>1/2 MS + 0.5мг/л IBA</td>
</tr>
<tr>
<td>3</td>
<td>MS8</td>
<td>1/2 MS + 1.0 мг/л IBA</td>
</tr>
<tr>
<td>4</td>
<td>MS9</td>
<td>1/2 MS + 0.5 мг/л IAA</td>
</tr>
<tr>
<td>5</td>
<td>MS10</td>
<td>1/2 MS + 1.0 мг/л IAA</td>
</tr>
</tbody>
</table>

### Results and Discussion

From the studies of the first group of clonal micropropagation, it was found that, for the introduction of explants in the culture, the best period at the beginning of growth and development and the phase of active growth and development had a regenerative capacity of more than 80-90%. To optimize the sterilization of the axillary buds, it was performed in several stages using three sterilizing agents with different exposure periods. It was found that the most effective sterilizing agent was sodium hypochlorite (90%) at exposition for 20 minutes, sterilization efficiency and yield of sterile viable explants was 92%.

By studying the second link of clonal micropropagation, the explants were planted on modified Murashige-Skuga nutrient media (MS), while the MS nutrient medium was modified four times (benzylaminopurine was added in different concentrations). All modifications contained the standard amount of macro- and micronutrients, sucrose and agar-agar, according to the Murashige-Skuga recipe. The pH of the medium was 5.6 to 5.8. The MS without phytohormones (MS 1) was used as a standard. At the concentrations of 0.5 mg/l and 1.0 mg/l cytokinins, namely BAP in most genotypes, no callus formation was observed, and the indices of new micro-sprouts and their length were the best.

### Rhizogenesis of Explants

After accumulation of a sufficient amount of vegetative material (Figure 1) under sterile conditions, it was rooted *in vitro*. The factors influencing the process of rooting *in vitro* were studied. This study focused on choosing the best type of auxin and its optimal concentration.
At the stage of Rhizogenesis induction (Figure 2) the frequency of rooting was studied (percentage of rooted micro-sprouts to the total number of micro-sprouts planted on the medium for Rhizogenesis).

The results of Rhizogenesis are presented in table 3. The number of rooted shoots is given. Comparing the two types of auxins and their concentrations, it was found that the best average rooting rates for rose varieties were 0.5 mg/l IBA and 1.0 mg/l IBA and 0.5 mg/l IAA. When using auxins, the frequency of rooting is much higher than without the use of phytohormones.
Considering the effects of phytohormones depending on variety, it should be noted that Gebruder Grimm, Lavaglut, and Friesia had the best rooting rates compared to MS6 (St) when using MS8 and was 87.56%, 82.11% and 94.33, respectively. The Tchaikovski variety had the best indices when using nutrient media MS7 – 98.44%, MS8 – 91.56% and MS10 – 95.11%. Coral Surprise variety had the best rooting frequency when using 0.5 mg/l IAA and was equal to 97.22%. In addition, high rates were observed when using 0.5 mg/l IBA and were equal to 96.67%. The lowest rates were at rooting without auxins and were equal to 16.00%. In the Alan Titchmarsh variety, the rooting frequency was the best among all varieties when using different types of auxins and concentrations and ranged between 91.89% and 99.00%.

Table 3: Frequency of rooting depending on the type of auxin and its concentration for different varieties of roses in vitro, %

<table>
<thead>
<tr>
<th>№ з/п</th>
<th>Variety</th>
<th>Modification MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS6(St)</td>
</tr>
<tr>
<td>1.</td>
<td>Gebruder Grimm</td>
<td>15.00</td>
</tr>
<tr>
<td>2.</td>
<td>Lavaglut</td>
<td>11.00</td>
</tr>
<tr>
<td>3.</td>
<td>Friesia</td>
<td>23.33</td>
</tr>
<tr>
<td>4.</td>
<td>Tchaikovski</td>
<td>21.56</td>
</tr>
<tr>
<td>5.</td>
<td>Coral surprise</td>
<td>16.00</td>
</tr>
<tr>
<td>6.</td>
<td>Alan Titchmarsh</td>
<td>27.89</td>
</tr>
<tr>
<td></td>
<td>Average value</td>
<td>19.13</td>
</tr>
</tbody>
</table>

The final stage of clonal micropropagation is the transfer of well-formed regenerating plants from in vitro to ex vivo conditions and their adaptation. The adaptation was carried out in several stages. At the first stage, the regenerating plants were formed more than 2 cm high with well-developed leaves and a well-developed root system, that were carefully removed from the tubes with tweezers with long ends. The micro-sprouts were washed from the nutrient medium and transferred to non-sterile substrates for adaptation with good aeration. Peat with perlite in the ratio (1:1) was used as a substrate; peat, soddy soil and perlite (1:1:1).

**Adaptation of Regenerating Plants**

At the first stage, an elevated (up to 90%) humidity level was maintained. Artificial fog conditions were created for better growth. After some time, the humidity level was gradually reduced. In addition, at this stage of adaptation, the effectiveness of adaptation was evaluated as a percentage of plants that took root in the substrate and began to form new leaves to the total number of plants planted in the soil (Figure 4).

Varieties of roses planted on the substrate peat, soddy soil and perlite (1:1:1) had a higher efficiency of adapted plants which reached 98% and it is more than the varieties planted on the substrate peat with perlite (1:1). However, for Gebruder Grimm and Lavaglut varieties, the best adaptation efficiency was shown when using peat substrate with perlite (1:1). The adaptation efficiency of these varieties was 87% and 85%, respectively. 20-30 days after planting, well-rooted plants were fed with a complex mineral fertilizer. Further cultivation of acclimatized plants corresponds to the accepted cultivation techniques.
Conclusion

In this study, the best average rooting rates for rose varieties were 0.5 mg/l IAA and 1.0 mg/l IAA and corresponded 91.24% and 90.15%, respectively. The best substrate for the first stage of rooting is peat, soddy soil and perlite (1:1:1). Using this substrate, the efficiency of adapted plants reached 98%. Thus, when optimizing the stages of clonal micropropagation in this study, the best genotypes for clonal micropropagation with the greatest Rhizogenesis are Alan Titchmarsh, Coral Surprise and Tchaikovski varieties.

References


Authors’ Declarations and Essential Ethical Compliances

Authors’ Contributions (in accordance with the ICMJE criteria for authorship)


<table>
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<tr>
<th>Contribution</th>
<th>Author 1</th>
<th>Author 2</th>
</tr>
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<td>Yes</td>
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<tr>
<td>Collected the data</td>
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<td>Yes</td>
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<tr>
<td>Contributed to data analysis &amp; interpretation</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Wrote the article/paper</td>
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<td>Critical revision of the article/paper</td>
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<td>Editing of the article/paper</td>
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<td>Supervision</td>
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<tr>
<td>Project Administration</td>
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<td>Funding Acquisition</td>
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<td>Overall Contribution Proportion (%)</td>
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<td>40</td>
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Has this research involved animal subjects for experimentation? No

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