RESEARCH ON MICROPROPAGATION OF GREEN ROSE (*Rosa* L.)

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SUMMARY

Green rose (*Rose* L.) is a beautiful, precious flower that is loved by many people all over the world. However, the source of seedlings of the ornamental flower variety is very limited. This study was conducted to rapidly propagate green roses by tissue culture. A solution of 0.1% $HgCl_2$ was used to sterilize the samples; some plant growth regulators such as BA, Kinetin, and nanosilver were used to improve the efficiency of *in vitro* propagation. The results showed that (*i*) the using the solution $HgCl_2$ 0.1% for sterilizing the shoots in 10 minutes gave a high rate of clean, survival samples, nearly 69%; (*ii*) the MS medium supplemented with 30 g/L sucrose, 8 g agar, 1.5 mg/L BAP, and 6 ppm nanosilver was the best for shoot regeneration with regeneration rate was 95.56%, and average shoot height was approximately 3 cm after 2 weeks of culturing, (*iii*) the most suitable medium for shoot multiplication was the MS medium supplemented with 30 g/L of sucrose, 8 g/L agar, 1.5 mg/L BAP, and 0.25 mg/L Kinetin with a coefficient of 2.65, and the average shoot height was 2.25 cm, (*iv*) the shoots can have the best induction of rooting as culturing in the MS medium added 2 mg/L α-NAA, and 2 ppm nanosilver with the rooting rate was 76.67%, and average root length was around 3.3 cm after 4 weeks culturing.

Keywords: green rose, *in vitro* micropropagation, nanosilver, plant grow regulator.

1. INTRODUCTION

Roses have been grown for a long time and associated with human's lives in various aspects such as romantic motels decorated with a bit of crimson, a little bright yellow, solemn places with peacock departments, partying, and gardens filled with beautiful color and fragrance. The green rose is a species of the genus Rosa (*Rosaceae* family) in which pigments range from blue to purple. With green color, although it only recently appeared, the spread and love of people for this flower is increasing quickly. It not only has a luxurious beauty but also carries many great meaningful values. Green roses are no exception with a high spiritual, aesthetic, and economic value.

In general, the demand for fresh flowers, especially flowers with beautiful and exotic colors like roses, is increasing significantly worldwide. However, there exists a difficult problem in breeding the new persimmon variety for the domestic market and export. One kind of green rose (*Rosa x odorata*), green aging to purplish green, collected in Bali Botanical Garden, was only studied on vegetative reproduction with quite a low efficiency (Siregar et al., 2005). Besides, it is said that *in vitro* propagation of roses could multiply rapidly cultivars with desirable traits and production of healthy and disease-free plants. During the last several years, scientists are witnessing several approaches for rose micropropagation. However, it is always challenging to find a suitable protocol and refinements with a high rate of shoot multiplication and a cost-effective method for a valuable variety (Rashida et al., 2003). Therefore, many findings of rose micropropagation have been reported for a long time, such as Nikbakht et al. (1560) to now (Siregar et al., 2005; Hameed et al., 2008; Naphaporn, 2009; Murali & Sindhu, 2011; Zeng et al., 2013; Nguyen & Van Le, 2020).

To develop the flower, the application of tissue culture could rapidly propagate this valuable green rose in a short time.

2. RESEARCH METHODOLOGY

2.1. Material: Green rose plants grown and preserved in the Vietnam National University of Agriculture.

Figure 1. Green rose plant

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2.2. Methods

2.2.1. Sample sterilization: According to Nguyen Ngoc Quynh Tho et al. (2018), $HgCl_2$ 0.1% was an appropriate chemical to sterilize rose plant samples. Therefore, before being treated by the $HgCl_2$ as the primary substance for sterilization, the segments carrying stems were washed under running water, soaked in a diluted soap for 15 minutes and then rinsed with distilled water 2 times in the sterilizing box, and rinsed the sample with 70˚ alcohol solution for 1 minute and lastly rinsed with distilled water. Specifically, the sample was sucked with $HgCl_2$ 0.1% in different periods like 5, 10, 15, or 20 minutes and cultured in the MS medium in one week. They were assessed with the survival rate (total number of survival samples/total samples × 100%) and the rate of clean survival samples (total number of clean survival samples/total samples × 100%).

2.2.2. Regeneration

a. Effect of BAP on shoot regeneration:

Nguyen Thi Phuong Thao (2015) used the MS medium supplemented with BAP for making up the highest rate of regeneration of rose samples in this stage. In this study, the MS medium supplemented with 8 g/L agar, 30 mg/L sucrose, and BAP (with different concentrations such as 0, 0.5, 1.0, 1.5, or 2.0 mg/L) was used to generate shoots *in vitro*. After 2 weeks, figure out the germination rate (the total number of germinated samples/total samples × 100%); the average height of shoot (cm).

b. Effect of silver on shoot regeneration

Ha Ngan Thi My et al. (2020) showed that silver nanoparticles could enhance the growth of *Rosa Hybrida L*. 'Baby Love' roses, and stimulate mass shoot propagation, rooting *in vitro*. Thus, the rose samples were cultured into the best medium of the previous experiment with BAP and supplemented with nanosilver (NS) with one of the following concentrations such as 0, 2, 4, 6, or 8 ppm. After 2 weeks, analyze the shoot multiplication coefficient (%), shoot's average height (cm).

2.2.3. Multiplication *in vitro*

a. Effect of BAP on shoots multiplication

The MS added 1.5 mg/L BAP was evaluated as the best medium for shoot proliferation of rose samples *in vitro* (Hameed et al., 2006; Bui Thi Thu Huong et al., 2017). Therefore, the MS medium supplemented with 8 g/L agar, 30 g/L sucrose, and BAP ranged from 0, 0.5, 1.0, 1.5, or 2.0 mg/L BAP. After 4 weeks, collect the shoot regeneration rate (total number of shoot regeneration samples/ total samples × 100%), shoot average height (cm).

b. Effect of Kinetin on shoots multiplication

The finding of our previous publications of *in vitro* multiplication of Sapa roses (*Rosa gallica L*.) (Bui Thi Thu Huong et al., 2017) illustrated that 91.67% of the Sapa roses' sample formed shoots on the culturing medium, the MS added 1.5 mg/L BAP and 0.5 mg/L Kinetin. The green rose shoots were also put on the MS medium and 8 g/L agar, 30 g/L sucrose supplemented with 1.5 mg/L BAP, and added Kinetin with the following different concentrations as 0, 0.25, 0.5, 0.75, or 1.0 mg/L. After 4 weeks, find out the shoot regeneration rate (total number of shoot regeneration samples/total samples × 100%); shoots average height (cm).

2.2.4. Rooting

In 2005, Nguyen Thi Kim Thanh et al. studied white roses' tissue culture and reported that MS medium supplemented with 2 mg/L α-NAA gave the rooting efficiency over 60%. Moreover, our previous finding of culturing the Sapa roses' shoots on MS medium supplemented with 2 mg/L α-NAA and 2 ppm NS demonstrated that the rooting rate increased with the rate of 76.67% (Bui Thi Thu Huong et al., 2017). It navigated our experiment in which *in vitro* rose shoots were cultured in the MS medium containing 2 mg/L α-NAA and added nanosilver with different concentrations like 0, 2, 4, 6, or 8 ppm. After 4 weeks, the root regeneration rate (total number of roots / a shoot × 100%); roots' average length (cm), and their main characteristics were analyzed.

2.2.5. Experiment condition and data analysis

The experiments were conducted at the Vietnam National University Agriculture, from 2020 to 2021. All the medium was adjusted to pH 5.8 and autoclaved at 1 atm, 121˚ in 20 minutes. The sample was illuminated with around 2500 lux in 16 hours at 25 ± 2˚C, humidity 70 - 80%. All experiments were repeated 3 times with 15 samples per formula each time, then observed and evaluated the figures for some weeks of culture depending on a particular situation. Data is processed according to software MICROSOFT EXCEL and statistical software IRRISTAT 5.0.

3. RESULTS AND DISCUSSIONS

3.1. Sample sterilization

To create *in vitro* materials of green rose plants, the stem segments were sterilized by $HgCl_2$ 0.1% in 5, 10, 15, or 20 minutes; then were cultured in the MS medium. The obtained results of sterilized samples a week later were shown in Table 1.

Table 1. Effect of $HgCl_2$ on nodal segments of green rose

Time (minutes)	Survival rate (%)	Clean, survival rate (%)
5	95.56	44.44
10	91.11	68.89
15	75.56	57.78
20	71.11	51.11

Table 1 shows that after 2 weeks of culturing, the percentage of survival samples sterilized at different times ranged from 71.11% to 95.56% and the clean survival rate from 44.44% to 68.89%. Among them, 10 minutes of treatment by $HgCl_2$ 0.1% led to the highest clean survival rate, 68.89%. It was quite like the research of Nguyen Ngoc Quynh Tho et al. (2018) in which, bud segments were disinfected by 0.2% $HgCl_2$ in 10 minutes, and the percentage of uninfected, survivals reached 71.67%.

Compared to the research of Banyal et al. (2015), who developed an efficient protocol for sterilizing axillary bud segments of *Rosa* × *hybrida* L. cv. Happiness with T1 pre-treatment comprising 0.2% Carbendazim, 0.2% Mancozeb-45, and 150 mg/L8-HQC in 4 radiation on a horizontal shaker (200 rpm), which resulted in 80.25% of survival explants. This experiment had similar findings when a different chemical, $HgCl_2$ 0.1% was used for rose explants' sterilization.

3.2. The possibility to regenerate *in vitro* shoots

The effect of BAP on stem segments' green rose to regeneration shoots was described in Table 2 after 2 weeks of culturing.

The results obtained in Table 2 and Figure 2 show that, after 2 weeks of culturing, the MS medium with 1.5 mg/L BAP made up the highest shoot regeneration rate, 88.67%, and the shoot height was also the highest, 2.05 cm.

Table 2. Effect of BAP on shoot regeneration of green roses' nodal segments

BAP (mg/l)	Regeneration rate (%)	Shoot height (cm)	Shoot characteristics
0	56.33^{d}	1.22^{d}	Small, green shoots
0.5	66.67^{c}	1.48^{c}	Small, green shoots
1.0	80.67^{b}	1.80^{b}	Quite big, green shoots
1.5	88.67^{a}	2.05^{a}	Big, green shoots
2.0	69.33^{c}	1.82^{b}	Big, light green shoots
$LSD_{0.05}$	6.70	0.23	
CV(%)	1.30	3.90	

In the same column, the value with the different letters was shown significantly differently at p = 0.05.

0 mg/L BAP *0.5 mg/L BAP* *1.0 mg/L BAP* *1.5 mg/L BAP* *2.0 mg/L BAP*

Figure 2. Shoots on the MS medium supplemented with BAP after 2 weeks of culturing

However, Duong Tan Nhut et al. (2015) declared that MS medium supplemented 7 mg/L nanosilver (NS) gave a better effect on their roses' sample than that without NS. The result was consistent with this experiment shown in Table 3, Figure 3 in which the medium with 6 or 8 ppm NS took the highest rate of shoot regeneration. However, the shoots in the medium with 8 ppm NS had yellow leaves. So, the medium with 6 ppm NS was optimal for the roses' shoot regeneration. Similarly, Ha Ngan Thi My et al. (2020) explained that NS could improve the growth of *Rosa Hybrida L*. 'Baby Love' in the different concentrations. Dong Huy Gioi and Duong Thi Men (2017) also reported that more than 91% of Sapa nodal explants formed shoots on the medium added 2 ppm NS.

Table 3. Effect of nanosilver on shoot regeneration of green roses' nodal segments

Nano silver (ppm)	Regeneration rate (%)	Shoot height (cm)	Shoot characteristics
0	84.44^{c}	2.1^{c}	Small, green shoots
2	86.67^{b}	2.4^{b}	Small, light green shoots
4	88.89^{b}	2.6^{a}	Quite big, light green shoots
6	95.56^{a}	2.8^{a}	Big, light green shoots
8	95.56^{a}	2.7^{a}	Big, light green shoots with yellow leaves
$LSD_{0.05}$	1.89	0.34	
CV (%)	1.20	6.9	

In the same column, the value with the different letters was shown significantly differently at p = 0.05.

0 ppm NS *2 ppm NS* *4 ppm NS* *6 ppm NS* *8 ppm NS*

Figure 3. Shoot on the MS medium supplemented 1.5 mg/l BAP and nanosilver (NS) after 2 weeks of culturing

3.3. Shoot multiplication

The *in vitro* shoots were cultured in the MS medium with BAP to multiply shoots. After 2 weeks, the data were collected and were shown in Table 4, and Figure 4 as well. After 2 weeks of culturing, the MS medium with 1.5 mg/L BAP was the best suitable to multiply shoots. It made up the highest shoot multiplication coefficient, 2.4, and the average shoot height was 2.02, and the number of new leaves of each shoot was 4.2. It was likely to be analogous to our research in 2017 on the Sapa roses (*Rosa gallica L.*), which illustrated the best result of multiplication as culturing in the MS medium added 1.5 mg/L BAP (Bui Thi Thu Huong et al., 2017).

Table 4. Effect of BAP on shoot multiplication of green roses' shoots

BAP (mg/L)	Shoot multiplication coefficient	Average shoot height (cm)	Number of leaves/ shoot	Shoot characteristics
0	1.06^{d}	1.30^{c}	2.7^{c}	Small shoots, green leaves
0.5	1.50^{c}	1.60^{b}	3.1^{bc}	Small shoots, green leaves
1.0	1.70^{b}	1.70^{b}	3.5^{b}	Quite big shoots, green leaves
1.5	2.40^{a}	2.02^{a}	4.2^{a}	Big shoots, green leaves
2.0	1.40^{c}	1.40^{c}	3.3^{b}	Quite big shoots, yellow leaves
$LSD_{0.05}$	0.24	0.19	0.36	
CV (%)	5.85	5.70	4.00	

In the same column, the value with the different letters was shown significantly differently at p = 0.05.

0 mg/L BAP *0.5* mg/L BAP *1.0* mg/L BAP *1.5* mg/L BAP *2.0* mg/L BAP

Figure 4. Shoot on the MS medium supplemented with BAP after 4 weeks of culturing

Besides, the MS medium with BAP and Kinetin would positively affect shoots in vitro to multiply. The experiment's results demonstrate the fact shown in Table 5, Figure 5, after 4 weeks of culturing. Although the MS medium with 1.5 mg/L BAP and 0.5 mg/L Kinetin was the most suitable for *Rosa indica L*. (Shabbir et al., 2009) and Sapa rose (Bui Thi Thu Huong et al., 2017), the results obtained in Table 5 and Figure 5 illustrates that the medium with 1.5 mg/L BAP and 0.25 mg/L Kinetin was optimal for these samples in multiplication *in vitro*. Specifically, the shoot multiplication coefficient was 2.65, and the average shoot height was 2.25, with the number of new leaves of each shoot reaching over 4.

Table 5. Effect of Kinetin on shoot multiplication of green roses' shoots

Kinetin (mg/l)	Shoot multiplication coefficient	Average shoot height (cm)	Number leaves/shoots	Shoot characteristics
0	2.07^{c}	2.03^{c}	3.6^{c}	Small, green shoots
0.25	2.65^{a}	2.25^{a}	4.3^{a}	Big, green shoots
0.50	2.30^{b}	2.14^{b}	4.0^{b}	Big, green shoots
0.75	2.33^{b}	2.07^{bc}	3.8^{b}	Big, yellow shoots
1.00	2.10^{c}	2.02^{c}	3.5^{c}	Big shoots, yellow leaves
$LSD_{0.05}$	0.24	0.10	0.27	
CV (%)	6.80	1.30	4.3	

In the same column, the value with the different letters was shown significantly differently at p = 0.05.

0.25 mg/L Ki *0.5 mg/L Ki* *0.75 mg/L Ki* *1.0 mg/L Ki* *1.25 mg/L Ki*

Figure 5. Shoots on the MS medium with 1.5 mg/L BAP and Kinetin (Ki) after 4 weeks of culturing

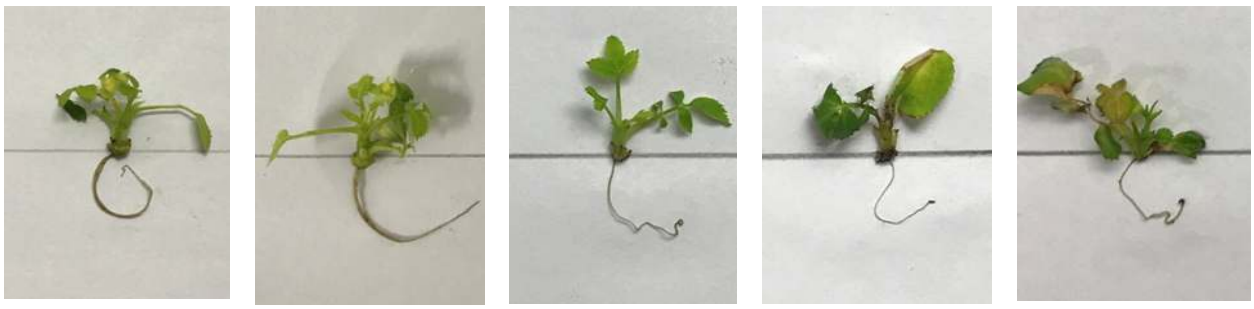
3.4. Rooting

According to Ha Ngan Thi My et al. (2020), nanosilver (NS) enhanced the growth of *Rosa hybrida* L. 'Baby Love and stimulated their rooting. It was likely to be similar for the green rose as well as when shoots were culturing in the MS medium with 2 mg/L NAA and NS at 2, 4, 6, or 8 ppm (shown in Table 6 and Figure 6).

Table 6. Effect of nanosilver on shoot rooting of green roses' shoots

Nanosilver (ppm)	Rooting rate (%)	Average root length (cm)	Roots' characteristic
0	60.00^{c}	2.24^{d}	Big roots
2	76.67^{a}	3.36^{a}	Quite big roots
4	64.33^{b}	2.94^{b}	Thin roots
6	56.67^{d}	2.66^{c}	Thin roots
8	46.67^{e}	2.42^{d}	Thin roots
$LSD_{0.05}$	1.93	0.27	
CV (%)	6.70	0.72	

In the same column, the value with the different letters was shown significantly differently at p = 0.05.



0 ppm NS *2 ppm NS* *4 ppm NS* *6 ppm NS* *8 ppm NS*

Figure 6. Shoots on the MS medium supplemented with 2 mg/L NAA and nanosilver after 4 weeks of culturing

The report of Nguyen Thi Kim Thanh et al. (2005) studying a white rose declared that MS medium augmented with 2 mg/L α-NAA gave the rooting efficiency over 60%. In this work, the green roses' shoots were cultured in the same medium and added 2 or 4 ppm NS had enlarged the rooting rates, 76.67%, and 64.33%, respectively; and the average root length was around 3 cm. In short, the ideal medium for rooting the green roses' shoots was MS medium added 2 mg/L α-NAA and 2 ppm NS.

4. CONCLUSION

Sterilizing samples for 10 minutes in $HgCl_2$ 0.1% was optimum to make clean survival samples with the highest rate, 68.89%. Besides, to regenerate new shoots, the MS added 6 ppm nanosilver and 1.5 mg/L BAP was ideal with the regeneration rate reaching 95.56%, average shoot height was 2.8 cm. The MS medium supplemented with 1.5 mg/L BAP and 0.25 mg/L Kinetin was the best suitable medium to multiply shoots *in vitro* as well, with the coefficient of 2.65, the average shoot height of 2.25 cm, and the number of leaves per shoot was around 4. In the MS medium including 2 mg/L α-NAA and 2 ppm nanosilver, 76.67% of shoots could form roots with an average root length of 3.36 cm.

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NGHIÊN CỨU VI NHÂN GIỐNG CÂY HOA HỒNG XANH (*Rosa* L.)

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TÓM TẮT

Hoa hồng xanh (*Rose* L.) là một giống hoa đẹp, mới lạ và được rất nhiều người ưa thích. Tuy nhiên, nguồn cây giống của giống hoa này còn rất hạn chế. Nghiên cứu này được thực hiện nhằm nhân nhanh giống hoa hồng xanh bằng phương pháp nuôi cấy mô tế bào. Dung dịch $HgCl_2$ 0,1% được sử dụng để khử trùng mẫu nuôi cấy, các chất điều tiết sinh trưởng như BAP, Kinetin và nano bạc được sử dụng để nâng cao hiệu quả của quá trình nhân giống *in vitro*. Kết quả thu được cho thấy (i) Sử dụng dung dịch $HgCl_2$ 0,1% để khử trùng mẫu trong 10 phút cho tỉ lệ mẫu sống sạchcao nhất, đạt gần 69%; (ii) môi trường MS có bổ sung 30 g/L sucrose, 8 g/L agar, 1,5 mg/L BAP và 6 ppm nano bạc là thích hợp nhất để tái sinh chồi hoa hồng xanh, với tỉ lệ tái sinh là 95,56% và chiều cao chồi khoảng 3 cm sau 2 tuần nuôi cấy, (iii) môi trường thích hợp nhất để nhân chồi là môi trường MS bổ sung 30 g/L sucrose, 8 g/L agar, 1,5 mg/L BAP và 0,25 mg/L Kinetin với hệ số nhân là 2,65 lần và chiều cao chồi trung bình là 2,25 cmsau 4 tuần nuôi cấy, (iv) chồi ra rễ tốt nhất khi nuôi cấy trong môi trường MS được bổ sung 2 mg/L α-NAA và 2 ppm nano bạc với tỉ lệ chồi ra rễ là 76,67% và chiều dài rễ trung bình khoảng 3,3 cm sau 4 tuần nuôi cấy.

Từ khóa: chất điều hòa sinh trưởng thực vật, hoa hồng xanh, nano bạc, vi nhân giống.

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