Effect of Growth Regulators on Rooting of Carnation (Dianthus caryophyllus) Tissue Culture Seedlings

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Abstract In order to understand the effects of different growth regulators and their concentrations on the rooting culture of carnation tissue culture seedlings, the optimal growth regulators and their concentrations were explored by adding different concentrations of IAA and IBA to the basic culture medium for tissue culture experiments and observing the rooting and growth of carnation tissue culture seedlings. The results showed that the sterilization effect of 10% sodium hypochlorite for 4 min was the best; the root length and number of roots in MS medium were significantly higher than that in 1/2 MS and 2 MS medium, and the roots were faster, the rooting rate was high, and the plant growth was good; IAA and IBA of 1.0 mg/L were all conducive to the root elongation, and significantly increased the number of roots, the rest of the concentration promoting effect was not significant, and the high concentration would inhibit the root growth. The growth of the plants treated with IBA was better, and the best was 1.0 mg/L IBA, which was most conducive to the rooting culture of carnation. The above results provide further theoretical basis for in vitro rapid propagation and industrial production of carnation cut flowers.

Keywords Carnation (Dianthus caryophyllus); Growth regulator; Rooting culture

Carnation (Dianthus caryophyllus) is a perennial herb of Caryophyllaceae in Dianthus L., which is named for beautiful flowers and fragrant aroma (Huang et al., 2004, China Agricultural Press, pp.4-5). It is mostly used for social interaction and environmental decoration (Ouyang et al., 2018). Carnation, which means warmth, enthusiasm and maternal love, is the most popular flowers on Mother's Day, and its demand ranks first among fresh cut flowers (Ma, 2017).

Plant growth regulators were discovered in the 1920s, and they have been synthesized artificially since the 1940s. Up to now, there are more and more kinds of plant growth regulators. Each plant growth regulator has its specific purpose, among which the rooting promoters include indole butyric acid (IBA), naphthalene acetic acid (NAA), indole acetic acid (IAA), 2,4-D and so on. Different target plants have different kinds of growth regulators, which are also affected by the concentration. Plant growth regulators have dual properties, which can promote growth at low concentration, but inhibit growth at high concentration. On the basis of previous studies (Hou et al., 2018), we explored the effects of different concentrations of growth regulators IAA and IBA on rooting of carnation tissue culture seedlings in this study, providing further theoretical basis for rapid propagation of cut flowers of carnation in vitro and industrial production.

1 Results and Analysis

1.1 Selection of sterilization time

When carnation explants were treated with 10% sodium hypochlorite for 2 min, the plants grew well, the leaves were green, and the incision was not obviously white, but a few plants were polluted. Sterilization for 4 min, the leaves were green, and the incisions were white, but there were no contaminated plants. At the later stage, the
plants returned to green, and the survival rate was high. Sterilization for 6 min, leaves were yellow, the incisions were white, and plant growth was bad (Figure 1).

![Figure 1 Comparison of sterilization effect of carnation explants](image1)

**1.2 Screening of basic medium**

The length and number of roots in MS medium were significantly higher than that in 1/2MS and 2MS medium ($p<0.05$), and the roots were faster, the rooting rate was high (Figure 2; Figure 3). In MS medium, the plant growth was the best, the number of roots was more, the roots were longer and thicker, and the leaves were green and flat. Although the plants in 1/2MS medium grew well, the number of roots was relatively small, and the roots were thin, the leaves were curly. The growth of plants in 2MS medium was poor, with slow rooting, low rooting rate, thin roots, and short plants (Figure 4).

![Figure 2 Effect of different concentrations of MS medium on root length](image2)

**Note:** Different letters indicate that the difference between treatments has reached 5% significant level.

**1.3 Effects of different growth regulators and levels on rooting of carnation seedlings in tissue culture**

**1.3.1 Effects of different growth regulators and levels on root length**

The root length of IAA at 0.5 mg/L and 0.8 mg/L was not significantly different from that of the control. At 1.0 mg/L, the root length was significantly higher than that of the control, while at 1.2 mg/L, the root length was significantly lower than that of the control. The root length of IBA at 0.5 mg/L, 0.8 mg/L, and 1.2 mg/L was not significantly different from that of the control. While at 1.0 mg/L, the root length was significantly higher than that of the control. Then, with the increase of IBA concentration, root elongation was inhibited (Figure 5).
Figure 3 Effect of different concentrations of MS medium on root number
Note: Different letters indicate that the difference between treatments has reached 5% significant level

Figure 4 Effects of different concentrations of MS medium on rooting and plant growth of carnation explants
Note: A,A': 1/2MS; B,B': MS; C,C': 2MS

Figure 5 Effects of different growth regulators and levels on root length
Note: Different letters indicate that the difference between treatments has reached 5% significant level
1.3.2 Effects of different growth regulators and levels on root number

The root number of IAA at 0.5 mg/L and 1.2 mg/L was not significantly different from that of the control. The root number of IAA at 0.8 mg/L was significantly lower than that of the control, while that at 1.0 mg/L was significantly higher than that of the control. The root number of IBA at 0.5 mg/L, 0.8 mg/L, and 1.2 mg/L was not significantly different from that of the control. While at 1.0 mg/L, the root number was significantly higher than that of the control. Then, with the increase of concentration, the number of roots decreased, but the number of lateral roots increased (Figure 6).

1.0 mg/L IAA and IBA could significantly promote root elongation, but there were significant differences in plant growth. The plant growth under different concentrations of IAA was bad, the stems were mostly bent, thin and weak, unable to grow upright, and the leaves were yellow and curly. While the plant growth of IBA was good. The plant growth of 1.0 mg/L IBA was better than that of the control, the stems were erect and strong, the leaves were dark green and extended. Both 1.0 mg/L IAA and IBA could significantly promote the growth of roots, but there were significant differences in plant growth (Figure 7; Figure 8).

![Figure 6](image6.png)

Figure 6 Effects of different growth regulators and levels on root number

Note: Different letters indicate that the difference between treatments has reached 5% significant level

![Figure 7](image7.png)

Figure 7 Effects of different concentrations of IAA on rooting and plant growth of carnation explants

Note: A,A': Control group; B,B': 0.5 mg/L IAA; C, C': 0.8 mg/L IAA; D,D': 1.0 mg/L IAA; E,E': 1.2 mg/L IAA
Among plant growth regulators, indole butyric acid (IBA), naphthalene acetic acid (NAA), indole acetic acid (IAA) and 2,4-D can promote plant rooting, which are commonly used in plant cutting propagation (Lin et al., 2019; Lu et al., 2020) and tissue culture (Xu et al., 2006; Xiao et al., 2016). However, different target plants have different suitable species and concentrations (Wei et al., 2017). Chai (2012) showed that 1/2MS+IAA 0.5 mg/L (or IBA 0.5 mg/L) was the best medium for root induction in carnation tissue culture. The results showed that although 1/2MS had fast rooting, the plant growth was not as good as MS medium. IAA and IBA can promote the rooting of carnation tissue culture seedlings in the appropriate concentration range, but there are some differences. 1.0 mg/L IAA and IBA were beneficial to root elongation and significantly increased root number. In the suitable concentration range, IBA has the most obvious promoting effect on rooting, and the longest root was 5.1 cm and the maximum number of roots was 19. While the longest root of IAA was 3.6 cm and the maximum number of roots was 14. The 1.0 mg/L IBA plant grew well and was the best for the rooting culture of carnation tissue culture seedlings. In previous experiments, the rooting rate reached 90% when NAA concentration was 1 mg/L, and there were significant differences in the number of roots and root length of explants compared with the control (Hou et al., 2018). The longest root length was 6 cm, and the maximum number of roots was 25, which was consistent with the research results of Yang (2005). In conclusion, IAA, IBA and NAA had promoting effects on the rooting of carnation tissue culture seedlings, with the order of NAA, IBA and IAA. And the appropriate concentration was 1.0 mg/L. The above results provide further theoretical basis on the effects of different combinations of growth regulators on the rooting culture of carnation tissue culture seedlings and the optimization of medium composition.

3 Materials and Methods
3.1 Experimental materials
Tissue culture seedlings were purchased from the market and potted in the laboratory. Stem segments of potted carnation master varieties without diseases and pests were selected as experimental materials.

3.2 Selection of sterilization time
Experimental design: Sterilization with 10% sodium hypochlorite for 2 min, 4 min and 6 min, respectively. The contamination rate and survival rate were counted 15 d after inoculation.
Culture conditions: The basic medium was MS with 3% sucrose and 0.6% agar powder, pH 5.8, culture temperature (25±1) °C, light intensity 2 000~2 500 lx, and light time 12 h/d. The explants were prepared according to the method of Hou et al. (2018). Each treatment was inoculated with 4 bottles, 3 plants in each bottle, and repeated 3 times. The pollution and growth were observed every day, statistics were made 15 d after inoculation.

3.3 Screening of basic medium
Experimental design: The medium of 1/2MS, MS and 2MS were respectively allocated, 3% sucrose and 0.6% agar powder were added, pH 5.8, culture temperature (25±1) °C, light intensity 2000~2500 lx, and light time 12 h/d. The statistics were made at 45 d.

3.4 Experimental design and method of different growth regulators treatment
Using MS as the basic medium, five concentration gradients of IAA and IBA were set, which were 0, 0.5 mg/L, 0.8 mg/L, 1.0 mg/L and 1.2 mg/L, respectively.

Inoculate with 300 mL bottles, 4 bottles per treatment, 3 plants per bottle, and repeat 3 times under the same culture conditions as above.

3.5 Statistical analysis of data
Pollution rate (%) = (number of pollution/total inoculation) × 100%
Rooting rate (%) = (number of rooting/total number of inoculation) × 100%
Survival rate (%) = (survival number / uncontaminated number) × 100%

The data of this experiment were statistically analyzed and plotted by Excel WPS 2019 and Duncan.a.b. difference analysis method of SPSS Statistics 17.0 (p<0.05).

Authors’ contributions
AQQG designed and carried out the study, and conceived of the project, directed the design of the study, data analysis, draft and revision. LJ performed the statistical analysis and drafted the manuscript. BXJL participated in the data analysis and drafted the manuscript. ZMD participated in the data analysis. ZYX participated in the design of the study and revision. All authors read and approved the final manuscript.

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