Effects of Gradient Concentration of Colchicine on Callus Induction and Differentiation of *Rose rosea*

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**Abstract.** To induce polyploid plants of *Rosa rugosa Thunb*, the effect of the interaction between the concentration of colchicine and the treatment time on the callus induction and differentiation of *R. rugosa thunb* was studied using colchicine as the inducer. The colchicine immersion method was selected, and the callus of red *Rosa rugosa thunb* was immersed in colchicine of different concentrations for 2h, 4h, and 8h. The best culture medium for inducing callus was as follows: MS+1.0mg/L 6-BA+0.1mg/L NAA+9.0 g agar+30.0g sucrose. After cultivation, the callus was dense in texture, light green and obviously granular. After the callus was immersed in colchicine, the combination of different colchicine concentrations and treatment time had an interaction with the callus. Therefore, the best treatment method for polyploid-induced *Rosa rugosa thunb* polyploid should be higher colchicine combined with shorter processing time. This experiment can provide experimental basis and theoretical basis for cultivating *R. rugosa thunb* polyploid plant with excellent traits, thereby increasing the content of essential oil in the plant itself.

1. Introduction

Plant essential oils are a class of aromatic substances with volatile oily liquids extracted from different organs such as leaves, flowers, roots, stems or fruits of higher plants [1]. They are secondary metabolites produced by many aromatic plants, such as *Ocimum basilicum*, *Melissa officinalis L.*, and *Rosa rugosa Thunb* [2]. *Rosa rugosa Thunb*, as an important ornamental and production double flower in the world, is one of the important special economic plants in China. The essential oil produced by the rose has a reputation of liquid gold [3]. According to the geographical and climatic classification, roses can be divided into tropical roses, temperate roses, and *Rosa rugosa thunb*, etc. and *Rosa rugosa* can grow normally in -40°C cold zone. *Rosa rugosa thunb* belongs to *Rosaceae*, which is commonly planted in the cold regions of northern China, northeast China, and Russia. Its petals can be used to make rose tea, raised sauce, and brewed rose wine, raised dew and other products. It has a wide range of applications and is also used in medical, beauty and essential oil production industries. Studies have shown that when rose is used for cutting and branch propagation, the growth period is long, and the disease is susceptible to disease during reproduction [4]. As a result, the amount of essential oils extracted from plants is very small, and it is difficult to meet the production needs of the perfume and essential oil industries, thereby restricting the development of the plant essential oil industry chain. Therefore, in order to increase the content of essential oils in plants, based on the shortage of raw materials, plaids breeding can effectively improve the genetic traits of haploid plants.
and expand their genetic resources. In this experiment, the leaves of *R. rugosa tunb* were used as experimental materials [5]. After the callus induction of the leaves, polyploid treatment was performed on them with colchicine to investigate the effects of different concentrations and treatment time of colchicine on the callus [6]. This will provide a guarantee for the cultivation of polyploid plants of *R. rugosa tunb* with excellent traits, and then increase the content of essential oil in the plant, laying a theoretical foundation for the study of alternative methods of traditional planting [7].

2. Materials and methods

2.1. Experiment material

Test materials: The *R. rugosa tunb* used in this experiment was introduced from Xinjiang and cultivated in Jiamusi University Science and Technology Park. Young leaves with good growth conditions and no pests were selected as experimental materials.

Main drugs: 6-BA (6-Benzylaminopurine), NAA (1-naphthyl acetic acid), IBA (Indole-3-Butyric acid), colchicine.

Main instruments: Vertical pressure steam sterilizer (DY04-13-44-00). Electric heating constant temperature drying oven (DUG-9247A) and clean workbench (JJ-CJ-2FD).

2.2. Experiment method

2.2.1. Screening of Optimum Media for Inducing Callus.

Select young leaves of *R. rugosa tunb*, immerse them in clear water containing surfactant for 20-30 minutes, and then rinse the active substances remaining on the surface of the leaves with running water; in a clean bench, put the washed leaves into 70% alcohol soak in medium for 30s, wash 4 times with sterile water; soak with 0.1% HgCl₂ solution for 8 minutes, wash with sterile water 4 times, and wash with sterile water for 30s-1min each time to complete the disinfection treatment. The leaves were cut into 0.5cm×0.5cm flakes, and the back of the leaves was inserted into the primary culture medium. The medium was prepared as follows:

1. MS + 2.5mg/L 6-BA + 0.2mg/L NAA + 9.0g agar + 30g sucrose;
2. MS + 1.0mg/L 6-BA + 0.1mg/L NAA + 9g agar + 30g sucrose;
3. MS + 0.1mg/L 6-BA + 0.5mg/L NAA + 9.0g agar + 30g sucrose;
4. MS + 1.5mg/L 6-BA + 0.1mg/L IAA + 7.0g agar + 30g sucrose;
5. MS + 1.0 mg/L 6-BA + 0.5mg/L NAA + 1.0mg/L 2,4-D + 9.0g agar + 30g sucrose, 4 tablets per bottle, 5 bottles per group, placed in constant temperature tissue culture with 2000lx light Cultivate on the shelf for about 30 days, and the light-dark ratio is 16: 8 (h), then callus can be obtained, and the state and callout rate of callus can be recorded.

2.2.2. Colchicine mutagenesis.

Callus tissues with good growth and no browning in the primary culture were immersed in solutions containing colchicine concentrations of 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, and at room temperature in a vertical thermostatic shaker, respectively. Shake for 2h; 4h; 8h, and rotate at 120r/min. The surface of the treated callus was cleaned, cut into a block shape of 0.5cm×0.5cm×0.5cm, and inoculated in a differentiation medium without colchicine (MS+2mg/L 6-BA+0.2mg/L IAA+7.0g agar+30.0g sucrose), the cut surface is brought into contact with the surface of the culture medium, 3 pieces per bottle, 10 bottles per group, repeat 3 times. Cultivate on a constant temperature tissue culture rack with a light intensity of 1600 lx at room temperature (25±3℃), alternately light and dark to 30 d, periodically observe its growth potential and growth status, and record data.

2.2.3. Data processing.

This experimental data was processed with Excel 2010 and analyzed by multivariate analysis of variance:

\[
C = \frac{\sum \text{Callus}}{S} \times 100\% \quad (1)
\]

\[
R = \frac{\sum \text{Death}}{S} \times 100\% \quad (2)
\]
Note: C represents the callout rate; R stands for the lethality; \( \Sigma \text{Callus} \) means the total number of callus growing from the leaves; \( \Sigma \text{Death} \) represents the total number of lethal; S stands for total leaf inoculation.

3. Results and analysis

3.1. Screening of Optimal Media for Callus Induction

The treated leaves were inoculated in MS medium containing different concentrations of plant growth regulators. After 15 days of incubation, the incisions of the leaves showed different degrees of swelling, with dark green tumour-like protrusions on the surface. After 30 days of cultivation, the shape of callus was obvious. It can be known that when 6-BA is 1.0mg/L, NAA is 0.5mg/L, and 2,4-D is 1.0 mg/L, the yield is 45%, the texture of the callus is loose, and the unevenness is obvious. The surface is green lustre. Without the addition of 2,4-D, CTK 6-BA was used as the leading factor for callus induction. Through comparison of callout ratios, it can be seen that the addition of excessively high concentrations of 6-BA has a relatively high growth of explants. The inhibitory effect reduced the growth state of the explants, and the healing rate was relatively reduced. In the D2 and D3 groups, when the concentration of 6-BA is the same, with the increase of the NAA concentration, the callus state is relatively loose, the callus rate has decreased by 34%, and the induction effect is reduced. Optimal medium for *R. rugosa tunb* callus induction: MS+1.0mg/L 6-BA+0.1mg/L NAA+9.0g agar+30g sucrose (In Table 1).

### Table 1. Effects of different plant hormone combinations on callus

| Handle | 6-BA (mg/L) | NAA (mg/L) | 2,4-D (mg/L) | Recovery rate | Staining rate | Growing state                  |
|--------|-------------|------------|--------------|---------------|---------------|-------------------------------|
| D1     | 2.5         | 0.2        | 0.0          | 48%           | 37.5%         | Dense, yellowish white, no obvious unevenness |
| D2     | 1.0         | 0.1        | 0.0          | 89%           | 0%            | Dense, light green, obvious bumps |
| D3     | 1.0         | 0.5        | 0.0          | 55%           | 0%            | Loose, green, obvious bumps   |
| D4     | 1.5         | 0.0        | 1.0          | 40%           | 0%            | Dense, green, uneven          |
| D5     | 1.0         | 0.5        | 1.0          | 45%           | 0%            | Loose, green, obvious bumps   |

3.2. Effects of different concentrations and treatment time of colchicine on callus

Colchicine acts on the centromere point to inhibit the formation of spindle filaments and double the chromosomes in the later stages of mitosis; however, the sensitivity of colchicine to different plant species, explant types and plant growth periods is significantly different. In the process, no obvious change was observed after 10 days of inoculation, and bud spots appeared after 25 days of culture. Multivariate analysis of variance was used to analyze the induction rate and lethality of callus *Rosa rugosa tunb* with different treatment time and concentration of colchicine, and the obtained products were morphologically identified.

### Table 2. Analysis of variance of colchicine induction rate

| Source of difference | SS   | df | MS  | F    | P-value | F crit |
|----------------------|------|----|-----|------|---------|--------|
| Processing time (h)  | 0.0576 | 4  | 0.0144 | 1.973955 | 0.191623 | 3.87853 |
| Processing concentration (mg/L)  | 0.126773 | 2  | 0.063387 | 8.689056 | 0.009875 | 4.45897 |
| Error                | 0.05836 | 8  | 0.007295 |         |         |        |

(1) Effect of single variable on callus induction and lethality of *R. rugosa tunb*. When the colchicine treatment time was the same, the induction rate and lethal rate of callus treated with different concentrations of colchicine had significant differences (P <0.05). Based on this, further comparison and analysis were performed based on this figure. The average mortality rate decreased first and then increased, indicating that within the test range of colchicine treatment concentration of 0.1-0.3 mg/L, the high concentration treatment resulted in an increase in callus mortality and a
significant adverse effect on callus. When the treatment concentration reaches 0.3 mg/L, the average mortality rate reaches a maximum of 37%, that is, within the concentration range of this experiment, there is an upper threshold for the effect of colchicine treatment concentration on the induction rate of callus of *R. rugosa tunb*. Compared with the treatment with low concentration colchicine, the callus size was reduced to varying degrees (in Table 3).

When the concentration of colchicine is the same, the effect of colchicine treatment time on its induction rate and lethality has no significant linear relationship. Under the same concentration in Figure 1, the induction rate gradually decreases with the increase of treatment time. As shown in Figure 2, the lethal rate is increasing with the treatment time. When the treatment time is 8h, the average lethality is as high as 40.2%, so the treatment time is too long to inhibit the growth of callus (in Table 2 and 3).

| Table 3. Variance analysis of colchicine mortality |
|--------------------------------------------------|
| Source of difference     | SS          | df | MS            | F          | P-value | F crit    |
| Processing time(h)       | 0.037293    | 4  | 0.009323      | 2.627525   | 0.114139| 3.837853  |
| Processing concentration (mg/L) | 0.071213    | 2  | 0.035607      | 10.03476   | 0.006598| 4.45897   |
| error                   | 0.028387    | 8  | 0.003548      |            |         |           |

(2) Effect of dual variables on callus induction rate of *R. rugosa tunb*. It was found that the combination of different concentrations and time of colchicine had an interaction effect on the callus induction rate. The induction rate of low concentration of colchicine and shorter treatment time was significantly higher than that of high concentration of colchicine and higher treatment time, but the induction rate did not reach the optimal value due to the treatment of low concentration of colchicine; therefore, comprehensive analysis can the combination of higher concentration of colchicine and shorter treatment time can make its induction rate reach the ideal value. In this experiment, the treatment time is 4 hours, and the concentration is 0.25 mg/L. The induction rate is 80%, and the average diameter is increased to 1.260 cm. Most of them are green non-embryogenic callus, and the growth state is strong (in Fig1 and 2).

4. Discussion and conclusion

4.1. Conclusion

After experimental research, the following conclusions were reached. The optimal medium for inducing callus was MS+1.0 mg/L 6-BA+0.1 mg/L NAA+9g agar+30.0g sucrose. The combination of higher concentration of colchicine and shorter treatment time is more suitable to affect *R. rugosa tunb* callus and there is an interaction effect[8].
4.2. Discussion

At present, colchicine is one of the most widely used chemical agents in polyploid species. Studies have shown that the concentration of colchicine and the treatment time have a serious effect on the success of polyploidy induction[9]; the treatment time is too short or the treatment concentration is low, the mitosis of meristem is difficult to synchronize, and chimeras are easy to obtain; too long treatment time and high concentration will make the callus affected by colchicine toxicity, so it will be based on the physiological characteristics of the experimental plant itself. Select the appropriate treatment time and treatment concentration to make the induction rate reach the best effect. In addition, different explant materials selected by different plants to induce polyploidy production will also affect the induction effect, such as seeds, stem segments, ex vivo tissues, clumps of buds, callus, etc. In this experiment, the young leaves and callus of *R. rugosa tunb* were used as the experimental materials. The callus was treated by the colchicine soaking method. During the cultivation process, some callus appeared browning and bacterial infection. Possible causes of such culture problems: colchicine itself is toxic and can inhibit or kill the growth of plant materials [10]; artificial factors such as improper aseptic operation and the like. Therefore, colchicine should be added cautiously in this study to prevent large-area pollution and browning, and lay the foundation for cultivating polyploidy *R. rugosa tunb* plants [11].

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