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Study on the Differences in Root System Development between Beihong and Muscat Hamburg Tissue Culture Seedlings

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Abstract: Beihong exhibits strong disease and frost resistance, allowing it to overwinter safely without soil burial in most wine-producing regions of China. It also possesses high fruit quality, making it suitable for winemaking. However, its rooting ability is poor in production. This study utilized tissue culture techniques, designing nine groups of tissue culture plantlets with varying concentrations of sucrose, NAA, and IBA through an orthogonal experiment to induce rooting. The Eurasian variety Muscat Hamburg, known for its ease of rooting, was used as a control to investigate the differences in rooting between the two varieties. The objective was to provide a theoretical basis for the propagation and rooting of Beihong in practical production. The number of main roots, number of lateral roots, and main root length for both varieties were measured at 20, 30, and 40 days after rooting treatment. Additionally, the levels of ABA, GA3, IAA, and tZ in the roots of both varieties were analyzed at five treatment stages (1, 2, 5, 6, and 8). The synthesis of IAA-related receptors and carrier-related genes in the roots was also quantified. The results indicated that Beihong exhibited a higher rooting rate and superior rooting index compared to Muscat Hamburg. Significant differences in endogenous hormone levels were observed between the roots of the two varieties. The correlation coefficient between the IAA level and root indices was high in Beihong but low in Muscat Hamburg. The expression levels of IAA-related genes in the root systems of both varieties showed considerable differences, though some similarities were also noted. Gene expression varied across the three observation periods. This study provides a theoretical foundation for improving the rooting process of Beihong.
Keywords: Wine grape; Tissue culture; Rooting; Endogenous hormones; Relative gene expression

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1. Introduction

Grapes are among the most widely cultivated fruit crops worldwide. Currently, most wine grape varieties grown in China are of Eurasian origin. Unlike in Europe, where they do not require burial for winter protection, some Chinese wine-producing regions experience a continental monsoon climate, necessitating soil burial for Eurasian grape varieties during winter. This practice significantly increases labor costs [1]. Therefore, the development of wine grape

varieties with enhanced cold and drought resistance, along with high fruit quality, is of particular importance. *Vitis vinifera-Vitis amurensis* Beihong (Beihong) is a hybrid of *Vitis amurensis* Rupr. and *Vitis vinifera* L. Muscat Hamburg. It possesses excellent disease and frost resistance, as well as superior fruit quality. Unlike most Eurasian varieties, Beihong can survive winter without soil burial in many wine-producing regions of China [2], making it highly suitable for winemaking. Addressing the challenges associated with Eurasian grape cultivation in China through variety selection is a promising approach.

This study employed tissue culture techniques to investigate the effects of different treatments on the rooting stage of Beihong. Comparisons were made with Muscat Hamburg, a Eurasian variety known for its superior rooting ability, to examine the differences in their rooting processes. The objective was to provide a theoretical basis for the propagation and rooting of Beihong in practical production [3].

2. Materials and methods

2.1. Test materials

The explants used in the tissue culture experiments for Beihong and Muscat Hamburg were biennial stems with uniform growth. In July 2021, bud-bearing stem segments of Beihong and Muscat Hamburg were collected from the wine grape experimental garden of "Ningxia Modern Agricultural Comprehensive Development Engineering Technology Research" in Pingjipu, Yinchuan, Ningxia. The experimental materials consisted of 7-year-old Beihong and Muscat Hamburg grapevines trained in a "factory" shape, with a plant spacing of 0.8 m × 3.0 m, oriented in a north-south direction [4].

2.2. Test methods

2.2.1. Sterilization of explants

Upon collection, healthy and uniformly growing explants were immediately selected and rinsed thoroughly with running water for 10 minutes. The explants were then disinfected in a 70% ethanol solution for 45 seconds under an ultra-clean workbench, followed by three washes with sterile water. Subsequently, they were sterilized with a 15% H_2O_2 solution for 6 minutes and rinsed four more times with sterile water [5].

2.2.2. Initiation of culture

For culture initiation, the sterilized stem segments with buds were trimmed at a 45° angle, approximately 5 mm from both ends. The lower end was placed in contact with the culture medium. The composition of the initial culture medium included MS basal medium supplemented with 1.0 mg/L 6-BA, 0.2 mg/L NAA, 30 g/L sucrose, and 7 g/L agar. The cultures were maintained under a 16-hour photoperiod at an illumination intensity of 2500 lx and a temperature of 25°C [6].

2.2.3. Rooting culture

Once the cultured stem segments developed shoots of approximately 5 cm, the uniformly growing shoots were excised under an ultra-clean workbench and transferred to rooting medium. Each bottle contained a single rootless seedling. The basic medium was 1/2 B5, supplemented with varying concentrations of IBA (0.1, 0.3, 0.5 mg/L), NAA (0.1, 0.3, 0.5 mg/L), and sucrose (15, 25, 35 g/L) in an orthogonal experimental design. The agar concentration was 7.5 g/L. The cultures were maintained at 25°C under a 12-hour photoperiod with an illumination intensity of 2500 lx. Each treatment was replicated with 10 samples, and the rooting rate was assessed after 20 days. The number of taproots, lateral roots, and the taproot length of tissue culture seedlings in each medium type were recorded at 20, 30, and 40 days after treatment [6].

2.2.4. Sample collection and measurement of root development

Samples were collected and photographed 20, 30, and 40 days after rooting treatment. For each treatment, 10 samples were analyzed to determine the number of taproots and lateral roots. The length of the taproots was measured using E-Ruler software. Rootless tissue culture seedlings were excluded from statistical analysis [7].

2.3. Data analysis

Data processing and visualization were performed using Excel 365 and Origin 2021 Pro. Statistical analyses, including one-way ANOVA, were conducted using SPSS 26.0, with LSD tests applied for multiple comparisons ($\alpha = 0.05$). Orthogonal test analysis was performed using Excel 365.

3. Results

3.1. Effects of different concentrations of IBA, NAA, and sucrose on the rooting rate of tissue-cultured red seedlings of Beihong and Muscat Hamburg

After 20 days of rooting treatment, the rooting rate of all treatments for Beihong exceeded 80%, indicating a better overall performance. For Muscat Hamburg, the lowest rooting rate was observed in treatment M8 at 60%, while the other treatments showed improved results. Treatments 4 and 6, both containing 25 g/L sucrose with a high concentration of NAA, resulted in a 100% rooting rate for both varieties [8].

3.2. Effects of different treatments on root development of tissue-cultured seedlings of Beihong and Muscat Hamburg

After 20 days of rooting treatment, the number of taproots in H6 was significantly higher than in H1, H5, and H7. The number of lateral roots in H4 was significantly higher than in H7. The taproot length of H7 was significantly greater than that of H2, H3, H4, H5, H6, and H8.

After 30 days, the number of taproots in H3 was significantly higher than in H5 and H7 [9]. The number of

lateral roots in H1, H2, H3, and H5 was significantly greater than in H7 and H9. The taproot length of H1, H2, and H3 was significantly longer than that of H4, H6, H7, and H8.

After 40 days, the number of taproots in H4 was significantly higher than in H1 and H9. The number of lateral roots in H1, H3, H4, and H8 was significantly greater than in H5, H7, and H9. The taproot length of H3 was significantly greater than that of H4, H5, H6, H7, H8, and H9.

3.3. Effects of different treatments on endogenous hormones in the root system of tissue-cultured seedlings of Beihong and Muscat Hamburg

After 20 days of rooting treatment, the GA3 content in H5 was the highest at 104.20 ng/g, significantly higher than in H1 and H2. No significant differences in tZ content were observed among treatments. The ABA content in H5 was 3 μg/g, significantly higher than in H2 and H6. The ABA content in H6 was 0.91 μg/g, significantly higher than in H5, while the other groups showed no significant differences.

After 30 days, the GA3 contents in H1 and H6 were 110.83 ng/g and 110.47 ng/g, respectively, significantly higher than in H2, H5, and H8. The tZ levels in H2 and H6 were 52.12 ng/g and 53.56 ng/g, respectively, significantly higher than in H1, H5, and H8. The ABA content in both H1 and H6 was 3.16 μg/g, significantly higher than in H2, H5, and H8. The IAA content in H2 was 0.87 μg/g, significantly lower than in other groups, while other differences were not significant.

After 40 days, the GA3 content in H5 was 104.44 ng/g, significantly higher than in H1, H2, and H6, while the lowest content was observed in H6 at 88.69 ng/g. The tZ content in H2 was 47.94 ng/g, significantly lower than in other groups. The ABA content in H5 was 2.99 μg/g, significantly higher than in H1, H2, and H6. The IAA levels in H2 and H6 were 0.94 μg/g and 0.98 μg/g, respectively, significantly higher than in other groups. Overall, hormone levels in H5 were highest after 20 days of rooting treatment, while H6 exhibited higher hormone levels after 30 days [10].

3.4. Effects of different treatments on the expression of IAA-related genes in the roots of Beihong and Muscat Hamburg

3.4.1. Effects of different treatments on the expression of IAA receptor-related genes in the roots of Beihong

After 20 days of rooting treatment, the relative expression levels of H2 *VvABP1* and H5 *VvTIR1* in Beihong were significantly higher than those observed in other treatments. After 30 days, the relative expression levels of H2 *VvABP1* and *VvTIR1* remained significantly higher in the rooting treatment compared to other treatments. Similarly, after 40 days, the relative expression levels of H5 *VvABP1* and *VvTIR1* were also significantly higher in the rooting treatment than in other treatments.

3.4.2. Effects of different treatments on the expression of IAA transporter-related genes in the roots of Beihong

After 20 days of rooting treatment, the relative expression levels of H2 and H5 *VvAUX1*, H8 *VvLAX1*, H2 *VvPIN1*, and H5 *VvABCB1* were significantly higher than those in other treatments. After 30 days, the relative expression levels of H1 *VvAUX1*, *VvLAX1*, H2 *VvPIN1*, and H2 *VvABCB1* in the rooting treatment remained significantly higher than in other treatments. By the 40th day, the relative expression levels of H5 *VvAUX1* and *VvLAX1*, as well as H2 and H6 *VvPIN1* and H2 *VvABCB1*, were still significantly higher in the rooting treatment than in other treatments.

3.4.3. Effects of different treatments on the expression of IAA receptor-related genes in the roots of Muscat Hamburg

After 20 days of rooting treatment in Muscat Hamburg, the relative expression levels of M6 *VvABP1* and M5 *VvTIR1* were significantly higher than those in other treatments. After 30 days, the relative expression levels of M8 *VvABP1* and M5 *VvTIR1* remained significantly higher than in other treatments. By the 40th day, the relative expression levels of M8 *VvABP1* and M6 *VvTIR1* in the rooting treatment were still significantly higher than those observed in other treatments.

4. Discussion

Recent studies have shown that NAA plays a crucial role in promoting cell division and expansion, inducing the formation of adventitious roots, and preventing fruit drop [11]. NAA treatment enhances the formation and establishment of adventitious roots, increases their number, improves the rooting rate, and shortens the rooting cycle. However, while it does not significantly affect the elongation of adventitious roots, it can substantially increase root dry weight, even up to ten times compared to treatments without NAA [12]. As a synthetic plant hormone, IBA facilitates root formation, cell elongation, and root growth [13].

Sucrose, a common carbon source in plant tissue culture, significantly influences plant growth. At a sucrose concentration of 20 g/L, the root length of honeysuckle remains unaffected, but in the absence of sucrose, both root length and plant height are reduced [14]. Similarly, for *Phalaenopsis* Pink, the optimal root number and length are observed at a sucrose concentration of 20 g/L [15].

In this study, we found that the optimal treatment for each root index of Beihong varied over time, whereas for Muscat Hamburg, the best results were consistently observed in treatment 6 (25 g/L sucrose + 0.5 mg/L NAA + 0.5 mg/L IBA). Orthogonal analysis indicated that sucrose concentration was the most significant factor affecting the rooting rate of both Beihong and Muscat Hamburg, with the optimal concentration being 25 g/L. The primary factor influencing the number of main roots was NAA concentration. For Beihong, the optimal concentration was 0.3 mg/L, while for Muscat Hamburg, it remained consistently at 0.5 mg/L. The number of lateral roots in Beihong was most affected by NAA concentration, with an optimal level of 0.3 mg/L, whereas sucrose concentration was the dominant

factor affecting lateral root formation in Muscat Hamburg, with an optimal concentration of 25 g/L. The key determinant of taproot length in Beihong was sucrose concentration, with an optimal level of 15 g/L, while for Muscat Hamburg, it was NAA concentration, with an optimal level of 0.1 mg/L.

5. Conclusion

Using an orthogonal design, we tested nine different combinations of sucrose, NAA, and IBA concentrations to induce rooting in tissue-cultured seedlings of Beihong and Muscat Hamburg. The results demonstrated that Beihong exhibited a higher rooting rate than Muscat Hamburg, though the root system of Muscat Hamburg developed more robustly. For Muscat Hamburg, the combination of 25 g/L sucrose + 0.5 mg/L NAA + 0.5 mg/L IBA consistently produced optimal rooting results. In contrast, the optimal culture medium for Beihong varied depending on the treatment duration. Analysis of endogenous hormone levels and IAA-related gene expression in roots at three stages revealed significant hormonal differences between Beihong and Muscat Hamburg, even under identical treatments. The correlation coefficient between IAA levels and root indices was high in Beihong but not significant in Muscat Hamburg. Furthermore, the expression of IAA-related genes varied considerably between the two cultivars, with correlations between gene expression and IAA levels fluctuating over time.

Disclosure statement

The author declares no conflict of interest.

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