Rooting and Transplanting Techniques for Vaccinium dunalianum Tissue-Cultured Seedlings (Postprint)

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Abstract

Vaccinium dunalianum Wight is one of the primary plant sources of arbutin, a natural whitening active ingredient widely utilized for skin whitening in the cosmetics industry. When employing plant tissue culture technology for seedling propagation, existing formulations result in callus formation at the base of rooted seedlings, thereby affecting transplantation success. To address the issues of poor rooting quality and low transplantation survival rates in V. dunalianum tissue culture seedlings, this study utilized subcultured seedlings as experimental materials. Single-factor experiments were conducted to screen optimal conditions for rooting, encompassing hormone type and concentration, basal medium type, and sucrose mass concentration. Additionally, the effects of different substrate compositions on the survival rate of transplanted V. dunalianum seedlings were investigated. The results demonstrated that hormone type and concentration, along with medium type, exerted the most significant influence on rooting rate, followed by sucrose mass concentration. The optimal hormone and concentration for V. dunalianum rooting was IBA at 2.0 mg · L ^{1}, the basal medium was 1/4 MS, and the sucrose mass concentration was 15 g · L ^{1}. The optimal rooting medium for V. dunalianum tissue culture seedlings was 1/4 MS + IBA 2.0 mg · L ^{1} + activated charcoal 0.1 g · L ^{1} + sucrose 15 g · L ^{1}, which achieved a 100% rooting rate with an average of 7.67 roots per plant. The root system developed radially without callus formation at the base, and the tissue culture seedlings exhibited vigorous growth with dark green foliage. For transplantation, pure humus soil substrate proved optimal, yielding an 83.7% survival rate with well-expanded leaves and robust growth. The optimized system established in this study effectively enhanced both the rooting rate and quality of V. dunalianum tissue culture seedlings, resolved post-transplantation survival difficulties, and provides a scientific foundation and technical support for the large-scale production of superior V. dunalianum plants.

Full Text

Preamble

Title: Rooting and Transplanting Techniques for Tissue-Cultured Plantlets of *Vaccinium dunalianum*

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Abstract

Vaccinium dunalianum Wight is one of the primary plant sources of arbutin, a natural skin-whitening active ingredient widely used in the cosmetics industry. During tissue culture propagation, existing formulations cause callus formation at the base of rooted plantlets, which compromises transplant survival. To address suboptimal rooting quality and low transplant survival rates in *V. dunalianum*, this study used subcultured plantlets as experimental material. Single-factor experiments were conducted to screen optimal conditions for rooting, including hormone type and concentration, basal medium type, and sucrose concentration. The effects of different substrate mixtures on transplant survival were also investigated. The results demonstrated that hormone type and concentration, along with basal medium type, had the greatest influence on rooting rate, followed by sucrose concentration. The optimal conditions for rooting were: IBA at 2.0 mg · L^{1}, 1/4MS basal medium, and sucrose at 15 g · L^{1}. The optimal rooting medium was 1/4MS + IBA 2.0 mg · L^{1} + activated charcoal 0.1 g · L^{1} + sucrose 15 g · L^{1}, achieving a 100% rooting rate with an average of 7.67 roots per plantlet. The root system was radially extended with no callus at the base, and plantlets grew vigorously with dark green leaves. For transplanting, pure humus substrate yielded the best results with an 83.7% survival rate, producing plants with expanded leaves and robust growth. This optimized system effectively improved rooting rate and quality while solving the challenge of low transplant survival, providing a scientific and technical foundation for large-scale production of superior *V. dunalianum* plants.

Keywords: *Vaccinium dunalianum* Wight, hormone, rooting, transplanting, tissue culture plantlet

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Introduction

Vaccinium dunalianum Wight, also known as "fanmiguo" or "changwei yueju," is a perennial evergreen shrub belonging to the family Ericaceae and genus *Vaccinium*. It naturally occurs in mountain shrublands, broad-leaved forests, or limestone scrub communities, occasionally growing as an epiphyte on evergreen broad-leaved trees or rarely as a small tree. The entire plant has medicinal value for dispelling wind-dampness and relaxing muscles and tendons. In China, it is mainly distributed in Yunnan, Sichuan, Guizhou, and Tibet (Fang Ruizheng, 1986). Our research group has identified *V. dunalianum* as a unique ethnic resource plant rich in arbutin and caffeoylarbutin compounds, making it one of the primary plant sources of the natural skin-whitening active ingredient arbutin widely used in cosmetics (Zhao et al., 2008). Young leaf buds of *V. dunalianum* are processed and consumed as a tea substitute among the Yi people in Yunnan. Due to their conical shape resembling bird beaks, they are called "quezui tea" (sparrow-beak tea). Studies have shown that both "quezui tea" and the air-dried fruits of *V. dunalianum* contain complete and abundant amino acids, along with rich mineral elements and nutrients, indicating high nutritional and health value (Yang et al., 2011; Luo et al., 2014b). The plant' s corolla is light green with purplish-red or light red hues, giving it ornamental value for cluster planting or as avenue trees in landscaping (Wang Lina, 2010), with broad prospects for urban greening applications.

Currently, *V. dunalianum* remains in the wild state. Combined with severe habitat destruction and slow plant growth, wild resources are becoming increasingly scarce, necessitating urgent development of germplasm conservation and improved breeding technologies. Common forest tree propagation methods include seed propagation, cutting propagation, and tissue culture propagation. Researchers using wild *V. dunalianum* seeds have investigated different treatments on seed germination, finding germination rates below 70% (Ren Guoxiang et al., 2017). Additionally, due to the lack of superior *V. dunalianum* families, seed propagation is prone to trait segregation. Traditional cutting propagation also has disadvantages including high scion requirements, significant damage to mother plants, and slow propagation rates.

Tissue culture rapid propagation offers an effective technology for *V. dunalianum* resource development and conservation. Tissue culture techniques have been reported for multiple *Vaccinium* species, with blueberry stem segments achieving 85.7% survival and 88% callus induction rates (Fang Xiaojing et al., 2014). He Jiawei and Xu Zhongzhi (2007) subcultured *Vaccinium duclouxii* buds on rooting medium, achieving 78% rooting rate and over 90% acclimatization survival. Our research group has established preliminary tissue culture protocols for *V. dunalianum*, including: (1) using young stem segments with buds from wild plants as explants to establish a tissue culture and rapid propagation system with 83.33% explant induction and 86.67% rooting rates (Luo Xulu et al., 2014a); and (2) using *V. dunalianum* tissue-cultured leaves to establish an adventitious root induction system for targeted production of arbutin and caffeoylarbutin secondary metabolites (Bu Chenghong et al., 2018). However, no studies have investigated transplanting and acclimatization of rooted plantlets. Furthermore, preliminary experiments found that while subcultured plantlets transferred to rooting medium achieved over 85% rooting rates, this was not optimal, and the existing rooting formulations produced extensive callus at the plantlet base, resulting in low transplant survival rates.

The quality of tissue-cultured rooting plantlets directly affects transplant survival rates and ultimately determines seedling production costs. Therefore, complete tissue culture propagation protocols require continuous optimization to reduce production expenses. To further improve the tissue culture rapid propagation process for *V. dunalianum*, reduce seedling costs, and advance commercial production, this study used subcultured plantlets as experimental material. We investigated hormone types and concentrations, basal medium selection, sugar concentration optimization, and substrate mixture ratios to enhance rooting rate, rooting quality, and transplant survival, providing technical support for superior *V. dunalianum* plant breeding and large-scale production.

Materials and Methods

1.1 Plant Materials

Subcultured plantlets derived from wild *V. dunalianum* collected in Wuding County, Yunnan Province, were provided by the Key Laboratory of Biodiversity Conservation in Southwest China, State Forestry Administration, Southwest Forestry University.

1.2.1 Experimental Material Pretreatment

Vaccinium dunalianum cluster buds were transferred to 1/2MS + IAA 1.5 mg · L ^{1} + activated charcoal 0.1 g · L ^{1} + sucrose 20 g · L ^{1} + agar 5 g · L ^{1} medium for vigorous growth treatment for two months to obtain robust subcultured plantlets and eliminate cytokinin effects. Plantlets with dark green leaves, vigorous growth, and no contamination were selected for subsequent rooting experiments. Medium pH was 5.8–6.0. Culture conditions were: temperature (25 ± 2)°C, light intensity 3000 lx, 12 h photoperiod. Rooting rates were recorded after 50 days. Experiments were conducted at the Key Laboratory of Forest Biotechnology in Universities of Yunnan Province, Southwest Forestry University.

1.2.2 Effects of Hormone Type and Concentration on Rooting

Using 1/2MS + agar 5 g · L ^{1} + activated charcoal 0.1 mg · L ^{1} + sucrose 15 g · L ^{1} as the basal medium, 12 treatments were established: IBA (1.5, 2.0, 2.5, 3.0 mg · L ^{1}), NAA (0.5, 1.0, 1.5, 2.0 mg · L ^{1}), and IAA (0.5, 1.0, 1.5, 2.0 mg · L ^{1}), plus a hormone-free control. Each treatment consisted of 30 bottles with 10 plantlets per bottle, repeated three times.

1.2.3 Effects of Basal Medium Type on Rooting

Five basal medium types were tested: MS, 1/2MS, 1/4MS, 1/8MS, and WPM, each supplemented with IBA 2.0 mg · L ^{1}, agar 5 g · L ^{1} + activated charcoal 0.1 mg · L ^{1}, and sucrose 15 g · L ^{1}. Each treatment consisted of 30 bottles with 10 plantlets per bottle, repeated three times.

1.2.4 Effects of Sugar Concentration on Rooting

Using 1/4MS + IBA 2.0 mg · L ^{1} + agar 5 g · L ^{1} + activated charcoal 0.1 mg · L ^{1} as the basal medium, four sucrose concentrations were tested: 5, 15, 25, and 35 g · L ^{1}. Each treatment consisted of 30 bottles with 10 plantlets per bottle, repeated three times.

1.2.5 Effects of Substrate Ratio on Transplant Survival

Robust rooted plantlets with expanded leaves and well-developed root systems were removed from culture vessels after 7 days of closed acclimatization. Residual medium was washed off, and plantlets were transplanted to acclimatization substrates. Four substrate mixtures were tested: 100% humus, red soil:humus (1:1), red soil:humus (2:1), and red soil:humus (4:1). All substrates were sprayed with carbendazim and watered thoroughly before transplanting the following day. Air temperature was maintained below 30°C. Each treatment transplanted 50 rooted plantlets, repeated three times. Survival rates were recorded after three months.

1.3 Data Analysis

Experimental data were processed using Excel 2007 and SPSS 19.0 software for statistical analysis. Rooting rate and survival rate were calculated as follows:

Rooting rate = (Number of rooted plantlets / Total number of inoculated plantlets) × 100%
Survival rate = (Number of surviving plantlets after transplanting / Total number of transplanted plantlets) × 100%

Rooting quality was visually assessed and categorized into four grades based on root system development and thickness: "excellent rooting" (++++), "good rooting" (+++), "poor rooting" (++), and "very poor rooting" (+).

Results

2.1 Effects of Hormone Type and Concentration on Rooting

As shown in Table 1 , the control group produced no roots. IAA treatments showed variable but non-significant rooting effects with few roots and slight callus formation at the base, achieving a maximum rooting rate of 73.33%. NAA treatments showed gradually increasing rooting rates with concentration, reaching 80.00% at 2.0 mg · L ^{1} NAA, with correspondingly vigorous and thick root systems, though root numbers remained low with extensive callus at the base. IBA treatments demonstrated significant concentration-dependent effects, with rooting rates initially increasing then decreasing as IBA concentration rose, peaking at 81.67% under 2.0 mg · L ^{1} IBA. This treatment produced optimal rooting with vigorous, thick, radially extended roots and no callus at the base, along with expanded leaves (Figure 1 [Figure 1: see original paper], Figure 2 [Figure 2: see original paper]).

2.2 Effects of Basal Medium Type on Rooting

Table 2 shows significant differences in rooting rates among different basal media. The 1/4MS medium produced the highest rooting rate at 97.22%, with numerous thick roots and vigorous plantlet growth (Figure 3 [Figure 3: see original paper], Figure 4 [Figure 4: see original paper]). The 1/2MS medium ranked second with an 81.67% rooting rate and good root numbers and plantlet growth. Other media produced thinner roots, lower rooting rates, and weaker plantlet growth.

2.3 Effects of Sugar Concentration on Rooting

Table 3 indicates that all sucrose concentrations from 5–35 g · L ^{1} produced relatively high rooting rates, with the 15 g · L ^{1} sucrose treatment achieving the maximum rooting rate of 100%. This treatment also produced the greatest root number and length, with radially extended roots, vigorous plantlet growth, and dark green leaves (Figure 5 [Figure 5: see original paper], Figure 6 [Figure 6: see original paper]).

2.4 Effects of Substrate Ratio on Transplant Survival

Table 4 shows that transplant survival rates of *V. dunalianum* rooted plantlets gradually decreased as the red soil proportion increased, with correspondingly poorer growth. Pure humus substrate proved optimal, achieving the highest survival rate of 83.64% with expanded leaves and robust growth (Figure 7 [Figure 7: see original paper]).

Discussion and Conclusion

This study investigated the effects of hormone type and concentration, basal medium type, and sucrose concentration on rooting induction in *V. dunalianum* tissue-cultured plantlets. The optimal rooting medium formulation was identified as 1/4MS + IBA 2.0 $\text{mg} \cdot \text{L}^{1}$ + activated charcoal 0.1 $\text{mg} \cdot \text{L}^{1}$ + sucrose 15 $\text{g} \cdot \text{L}^{1}$, achieving a maximum rooting rate of 100%. Further investigation of substrate mixtures revealed that pure humus substrate was optimal for transplanting, with an 83.7% survival rate.

Rooting and acclimatization represent the two final core stages in plant tissue culture rapid propagation. Rooting quality directly affects subsequent acclimatization success, while transplant survival rates directly influence production costs. During rooting, hormone type and concentration are most critical for root formation. Ikeuchi et al. (2016) found that plants can regenerate missing tissues and organs, with hormone treatment accelerating this process. Auxin effects on adventitious root induction vary among plant species (Abdulaziz & Bahrany, 2002). Auxins IBA and NAA demonstrate more stable rooting induction than IAA (AI-Juboory et al., 1998), with numerous studies confirming IBA' s superior effectiveness across many species (Prakash et al., 1999; Rani & Grover, 1999; Fracro & Echeverrigaray, 2001). This study compared IAA, NAA, and IBA effects on *V. dunalianum* rooting, finding that IAA and NAA treatments produced extensive callus at the plantlet base, whereas IBA treatment yielded callus-free, high-quality rooting. The rooting rate initially increased then decreased with rising IBA concentration, similar to patterns observed in *Michelia skinneriana* (Cao Jiwu et al., 2015), blueberry (Han Dewei, 2013), and *Vaccinium uliginosum* (Wang Zhenwu et al., 2015). The optimal IBA concentration of 2.0 $\text{mg} \cdot \text{L}^{1}$ for *V. dunalianum* plantlet rooting was higher than the 1.0 $\text{mg} \cdot \text{L}^{1}$ optimal for leaf-derived adventitious rooting in our previous study (Bu Chenghong et al., 2018), suggesting differential sensitivity to IBA between stems and leaves. This study only examined single hormone effects; future research should investigate hormone combinations to further improve rooting quality and reduce production costs.

Macronutrients play crucial roles in plant growth and development. Beyond nitrogen, elements including phosphorus, potassium, calcium, and magnesium influence plant cell enzyme activities and metabolic processes. Cheng Lei and Hu Songying (2003) found that low Ca^{2} and Mg^{2} concentrations promote root formation and growth. This study showed that 1/4MS medium produced the highest rooting rates and quality, while MS medium inhibited rooting, likely due to high macronutrient concentrations (e.g., Ca^{2} and Mg^{2}) suppressing root formation. Zheng Xiaojiang and Liu Jinlong (2001) similarly reported that high inorganic salt concentrations inhibited rooting in *Aesculus wilsonii*. The reduced rooting rate in 1/8MS compared to 1/4MS suggests that excessively low macronutrient concentrations cannot meet nutritional requirements for rooting, consistent with Liu Zheng' e et al. (2012). Previous work by Luo Xulu et al. (2014a) identified WPM as optimal with 86.67% rooting, whereas this study found only 12.78% rooting in WPM with poor root growth, possibly due to the vigorous growth pretreatment applied before rooting in the current study. The underlying mechanism requires further investigation.

Sucrose serves as a carbon source and energy supply for tissue-cultured plantlets while regulating osmotic pressure and enhancing salt tolerance (Liu Liping, 2006). Widely used in plant tissue culture, sucrose provides energy for growth and promotes tissue differentiation. Studies report variable sucrose effects on root growth: some found high sucrose concentrations promoted adventitious rooting (Yan Huabing et al., 2011), while others reported inhibition in wheat (Zhang Shuo et al., 2012) or minimal effects in *Dendrobium nobile* (De Faria et al., 2004). This study found that all sucrose concentrations produced rooting rates above 85%, indicating minimal sucrose influence on *V. dunalianum* rooting. Thus, sucrose effects on rooting are species-specific. Activated charcoal in rooting medium adsorbs toxic substances secreted by plant cells and harmful compounds in the medium while creating a dark environment similar to natural conditions, thereby promoting rooting (Liu Genlin et al., 2001; Zhang Wenquan et al., 2015; Feng Hanqing et al., 2016).

Vaccinium species have weak root systems lacking lateral roots, making transplant survival challenging (Liang Xiaojing et al., 2011) and imposing high substrate requirements. During transplanting, substrates support and anchor plants while providing suitable growth environments. Different substrates vary in moisture retention, aeration, and antimicrobial properties. As *V. dunalianum* belongs to the same genus as blueberry (Dong Chaoli, 2011), cranberry (Zhang Lihua, 2012), and lingonberry (Tian Xinhua et al., 2015), it similarly thrives in loose, porous, acidic artificial substrates rich in organic matter. This study demonstrated that pure humus substrate produced the best transplant results with 83.7% survival, likely due to its rich organic content and loose, aerated texture favorable for seedling growth. Additionally, since tissue-cultured plantlets transition from high-humidity, constant-temperature sterile conditions to low-humidity, variable-temperature non-sterile environments, they have weak adaptability (Huang Zhuozhong and Yan Huabing, 2007). Consequently, environmental conditions must be strictly controlled. Beyond routine substrate disinfection, post-transplant management is critical; temperature and humidity must be carefully regulated to prevent seedling mortality.

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Note: Figure translations are in progress. See original paper for figures.