Effects of Exogenous Hormone Treatment on Root Development of Actinidia Arguta Tissue-Cultured Seedlings

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Abstract: Using the elite variety "Longcheng No. 2" of *Actinidia argut*a as the experimental material, this study aimed to investigate the effects of different concentration combinations of naphthalene acetic acid (NAA) and 6-benzylaminopurine (6-BA) on root induction in *Actinidia arguta* cuttings. The goal was to screen out the optimal hormone ratio, providing theoretical basis and economic value for its tissue culture and rapid propagation. Results showed that Treatment Group I (NAA 0.10 $mg \cdot L^{-1}$ + 6-BA 2.00 $mg \cdot L^{-1}$) exhibited the best performance in terms of rooting rate, average number of roots, and root length. Specifically, its rooting rate reached 94.00%, with an average of 3.50 roots per cutting, an average root length of 5.60 cm, an average root diameter of 1.80 mm, and the longest main root reaching 10.5 cm, which were extremely significantly higher than those of the control group (CK) ($P < 0.01$). Treatment Group H (NAA 0.05 $mg \cdot L^{-1}$ + 6-BA 2.00 $mg \cdot L^{-1}$) achieved a rooting rate of 75.00%, with an average of 3.00 roots, an average root length of 3.10 cm, and an average root diameter of 0.90 mm. Treatment Group G (NAA 0.01 $mg \cdot L^{-1}$ + 6-BA 2.00 $mg \cdot L^{-1}$) showed a rooting rate of 56.30%, with an average of 3.00 roots, an average root length of 1.80 cm, and an average root diameter of 0.90 mm, both of which were significantly superior to CK ($P < 0.05$). Treatment Group F (NAA 0.10 $mg \cdot L^{-1}$ + 6-BA 1.50 $mg \cdot L^{-1}$) had the same average number of roots as Group I (3.50 roots) but with a root length of only 0.90 cm. The medium and low concentration groups (B, C, D, E) showed mediocre performance, with the average number of roots and root length mostly remaining below 1 root and 0.8 cm, respectively, with no significant differences observed. Group A (lowest concentration) had the poorest rooting effect, and the CK group failed to root throughout the experiment. In conclusion, the concentration combinations of NAA and 6-BA significantly affect the rooting of *Actinidia arguta* cuttings, with the ratio in Group I showing the optimal effect. This study provides theoretical basis and technical support for the rapid propagation of *Actinidia arguta*, and holds important practical significance for the large-scale cultivation and promotion of its elite varieties.

1. Introduction

Despite the abundant resource base and wide distribution of *Actinidia arguta* in China, a large-scale planting system has not yet been established. With the continuous growth of market demand for *Actinidia arguta* products, the demand for high-quality seedling supply has increased significantly. Wild *Actinidia arguta* has low natural reproduction efficiency; seed propagation has a long cycle, and offspring traits are prone to segregation, which is unfavorable for the stable preservation of excellent traits. Conventional cutting propagation is highly affected by environmental factors with a low propagation coefficient, making it difficult to achieve large-scale and rapid propagation. Therefore, tissue culture can maximize the preservation of the excellent traits of the mother plant and enable rapid propagation, allowing the acquisition of a large number of superior plant materials in a short period [1]. Using "Longcheng No. 2" as the experimental material to explore its root induction characteristics under different hormone ratios not only helps screen suitable hormone combinations for proliferation culture and root promotion but also provides academic references for establishing standardized and efficient in vitro propagation technologies. Among them, the status and parameters

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of plant roots play a crucial role in practical production and scientific experiments, directly affecting the yield and quality of seedlings. Therefore, screening the optimal hormone ratio is of great research significance and value for *Actinidia arguta.*

Plant tissue culture, as an efficient asexual propagation method, can be used to construct a rapid and efficient seedling propagation system, and it is one of the important technical approaches for seedling propagation of *Actinidia arguta*. Tissue culture technology enables the rapid cultivation of superior seedlings with a high propagation rate [2]. In China, tissue culture of *Actinidia arguta* began in the 1990s; Li Wei et al. first conducted tissue culture propagation of autumn buds of *Actinidia arguta* [3], and subsequent scholars have studied various varieties of *Actinidia arguta* [4]. In the tissue culture process of *Actinidia arguta*, the root induction stage is often a key link affecting the plant survival rate and transplanting success rate. The types and concentration ratios of exogenous plant hormones are critical factors influencing induction effects, mainly reflected in cell division and growth, tissue and organ differentiation, plant germination and rooting, and in vitro tissue culture [5]. At present, naphthalene acetic acid (NAA), as a widely used synthetic auxin, can effectively promote the formation and growth of adventitious roots in plants, with good root-promoting effects [6]. 6-benzylaminopurine (6-BA), as a cytokinin, plays a role in promoting cell division and differentiation, coordinating root and bud development [7], stimulating plant growth and metabolism, improving flowering development [8], and delaying leaf senescence. Previous studies have shown that a reasonable ratio of NAA and 6-BA not only helps improve the root induction rate of *Actinidia arguta* but also may optimize root structure and physiological activity, laying a foundation for the good growth of plants after transplanting. Clarifying the root induction mechanism and optimal ratio of these two hormones in the proliferation culture stage is one of the key research topics in current tissue culture studies of *Actinidia arguta*, which can optimize the experimental process and improve plant propagation efficiency.

Therefore, this experiment uses "Longcheng No. 2" *Actinidia arguta* as the experimental material to systematically and scientifically investigate the effects of different concentration combinations of NAA and 6-BA on its root induction. The purpose is to screen out the optimal hormone ratio, providing support for the subsequent establishment of an efficient and stable in vitro propagation system, and promoting the large-scale propagation and rapid propagation technology of high-quality *Actinidia arguta* seedlings.

2. Materials and Methods

The experiment used "Longcheng No. 2" Actinidia arguta plants collected from the Actinidia arguta germplasm nursery of Liaoning Agricultural Vocational and Technical College. All plant materials were healthy adult mother plants with robust growth, no pests or diseases, and stable genetic traits.

2.1. Reagents and Instruments

Reagents included MS medium, 6-BA, NAA, sucrose, agar powder, ethanol, and distilled water. The MS medium contained macroelements (NH_4NO_3, KNO_3, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$), microelements (e.g., H_3BO_3, $MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$, KI), iron salt stock solution, and organic components (including Organic 1: thiamine hydrochloride, nicotinic acid, pyridoxine hydrochloride; Organic 2: inositol; Organic 3: glycine).

Experimental instruments mainly included: an electronic balance (0.01 g precision), pH test strips, magnetic stirrer, autoclave, ultra-clean workbench, glass Petri dishes, graduated cylinders, beakers, conical flasks, forceps, scissors, vernier calipers (0.01 mm precision), steel tape measure (0.1 cm precision), sterile gloves, masks, pipette tips, and pipettes.

2.2. Experimental Design and Treatment Setup

A full factorial design of plant growth hormones (NAA and 6-BA) was adopted in this study. Sterile stem segments of "Longcheng No. 2" Actinidia arguta were used as explants to explore root induction effects under different hormone ratios. NAA concentrations were set at 0.01, 0.05, and 0.10 $mg \cdot L^{-1}$,

and 6-BA concentrations at 1.0, 1.5, and 2.0 mg·L⁻¹, resulting in 9 treatment groups, with a blank control (CK) included. Each treatment was replicated 4 times, with 4 stem segments inoculated per replicate. The experimental culture period was 55 days. Details of the treatment groups are shown in Table 1.

Table 1 Experimental Treatment Groups

Treatment	NAA (mg·L⁻¹)	6-BA (mg·L⁻¹)
CK	0.00	0.00
A	0.01	1.0
B	0.05	1.0
C	0.10	1.0
D	0.01	1.5
E	0.05	1.5
F	0.10	1.5
G	0.01	2.0
H	0.05	2.0
I	0.10	2.0

Note: Hormone concentration ratios for each treatment group.

2.3. Experimental Methods

2.3.1. Material Preparation and Sterilization

Annual, healthy, pest-free semi-lignified shoots of Actinidia arguta were selected, and 10 cm cuttings were taken, each retaining 2–3 leaves. All materials were sterilized within 4 hours of collection to minimize water loss and physiological activity decline. For pre-treatment, materials were rinsed with running tap water for 5 minutes to remove surface attachments. An optimized explant sterilization method was then used: immersion in 0.1% sodium hypochlorite for 5 minutes followed by disinfection with 75% ethanol for 1 minute, after which subsequent culture proceeded.

2.3.2. MS Medium Preparation and Sterilization

MS medium was used as the basal medium. First, sucrose (30 g·L⁻¹) and agar (7 g·L⁻¹) were added according to the standard formula. The medium was then heated and thoroughly stirred using a medium boiling device, followed by the addition of macroelements, microelements, and organic components to ensure full dissolution and mixing. The pH was adjusted to 5.5–5.8. Finally, plant growth regulators (6-BA and NAA) at the required concentrations were added to different treatment groups according to the experimental design. The medium was dispensed into sterilized glass Petri dishes (30 mL per dish), labeled with corresponding treatment codes, and sterilized in an autoclave at 121 ℃ for 20 minutes. After sterilization, the medium was cooled to room temperature before inoculation.

2.3.3. Inoculation Operation

Before inoculation, the inner walls of the ultra-clean workbench and operating tools were thoroughly wiped with 75% ethanol, and ultraviolet sterilization was performed for 30 minutes to ensure a sterile environment. Operators wore sterile gloves and masks, which were disinfected with alcohol before inoculation. Plant stem segments were cut to a length of approximately 1.0 cm with flat incisions, retaining 1 bud and 1 leaf. Finally, the cut stem segments were inserted into the medium using sterile forceps, ensuring they were securely fixed in the agar.

2.3.4. Culture Conditions

Cultivation was conducted in a constant-temperature artificial climate chamber under the following conditions: temperature (25 ± 1) ℃, light intensity 2000–2500 lux, and a photoperiod of 16 h light/8 h dark.

2.4. Data Collection

After the first signs of rooting, all cuttings were observed every 5 days by treatment group, and

root conditions were recorded. The measured indicators included:

Rooting rate (%) = (Number of rooted cuttings / Total number of cuttings) × 100

Average number of roots = (Number of main roots + Number of lateral roots) / Total number of roots

Root length (cm): Measured using a steel tape measure with 0.1 cm precision.

Root diameter: Measured at the base of the main root using vernier calipers (0.01 mm precision), expressed as the average value.

2.5. Data Analysis

SPSS 29.0 software was used to perform one-way analysis of variance (ANOVA) on data from each treatment group. A significance level of $P < 0.05$ was used to test for significant differences between indicators.

3. Results and Analysis

3.1. Effects of Different Hormone Combinations on Rooting Rate

Significance analysis showed that different concentration combinations of NAA and 6-BA had a significant effect on stem rooting of Actinidia arguta during proliferation culture ($P < 0.05$). As shown in Table 2, the rooting rate gradually increased with the increase in NAA and 6-BA concentrations. Among all treatments, Treatment I (NAA 0.10 mg·L^{-1} + 6-BA 2.00 mg·L^{-1}) achieved the highest average rooting rate of 94.00%, which was significantly higher than other treatment groups, making it the optimal treatment in this experiment. Then came the treatment with H (NAA 0.05 + 6-BA 2.00 mg/L), which had a rooting rate of 75.00% and showed excellent growth conditions and trait indicators, significantly outperforming the other groups. In addition, Treatment G (0.01 + 2.00 mg·L^{-1}) and Treatment C (0.10 + 1.00 mg·L^{-1}) also showed relatively good root induction effects, with rooting rates of 56.30% and 44.00%, respectively, and could be considered as suboptimal combinations.

However, low-concentration hormone combinations such as Treatments A (0.01 + 1.00 mg·L^{-1}) and B (0.05 + 1.00 mg·L^{-1}) had low rooting rates of only 6.30% and 19.00%, respectively, which were much lower than those of medium- and high-concentration groups, indicating limited root-inducing effects. The blank control group (CK) without any hormone addition had a rooting rate of 0.00%, showing extremely significant differences from all hormone-treated groups. Therefore, exogenous application of NAA and 6-BA plays an important and significant role in promoting rooting during the proliferation culture stage of Actinidia arguta.

In summary, different hormone concentration treatments had significant effects on stem rooting of Actinidia arguta, with significant differences between treatments ($P < 0.05$). Thus, the ratio of Group I (NAA 0.10 mg·L^{-1} + 6-BA 2.00 mg·L^{-1}) was the optimal hormone combination, as it significantly improved the rooting rate of Actinidia arguta stems and should be prioritized in practical in vitro rapid propagation. These results provide a theoretical basis for large-scale seedling production and efficient cultivation of Actinidia arguta, and help to further optimize rooting efficiency during proliferation culture.

Table 2 Effects of Different NAA and 6-BA Concentrations on Rooting Rate of Actinidia arguta

Treatment	NAA (mg·L^{-1}) + 6-BA (mg·L^{-1})	Number of Cuttings	Number of Rooted Cuttings	Rooting Rate (%)
A	0.01 + 1.00	16	1	06.30 ±12.50 fg
B	0.05 + 1.00	16	3	19.00 ±23.94 fg
C	0.10 + 1.00	16	7	44.00 ±23.94 cd
D	0.01 + 1.50	16	6	38.00 ±25.00 de
E	0.05 + 1.50	16	4	25.00 ±28.90 ef
F	0.10 + 1.50	16	6	38.00 ±14.43 de
G	0.01 + 2.00	16	9	56.30 ±12.50 bc
H	0.05 + 2.00	16	12	75.00 ±00.00 ab
I	0.10 + 2.00	16	15	94.00 ±12.50 a
CK	0.00 + 0.00	16	0	00.00 ±00.00 g

Note: Different lowercase letters in the same column indicate significant differences at the 0.05 level ($P < 0.05$). The same applies below.

3.2. Effects of Different Hormone Combinations on Root Number and Growth Trend

Statistical analysis of experimental results showed that different concentration combinations of NAA and 6-BA led to significant differences in root number and overall root growth trends of Actinidia arguta stems. The CK group showed almost no rooting, indicating that effective rooting of cuttings requires induction by plant hormones. In addition, the low-concentration Treatment A exhibited slow rooting with low root quantity, with an average of only 0.30 roots per cutting. Rooting initiated around day 30 of the culture period, and the peak rooting period occurred between days 40–50, indicating low root induction efficiency under low hormone concentrations.

With increasing concentrations of NAA and 6-BA, the total number of roots showed an overall increasing trend. The medium-low concentration groups (B, C, D, E) had an average root number ranging from 1.00 to 1.50, which was slightly higher than that of the low-concentration group but with no significant differences between groups. Treatments D and E showed average rooting performance in the early growth stage, with significantly fewer roots compared to medium-high concentration groups, but their growth rate accelerated significantly in the middle and late stages. Rooting initiated between days 30–35 of the culture period, with the peak period between days 40–55, indicating that plant hormones within this concentration range had limited effects on promoting tissue differentiation and growth.

The medium-high concentration groups (F, G, H, and I) exhibited obvious advantages in root promotion. Groups G and H had an average of 3.00 roots per cutting, with rooting initiating between days 20–25 and maintaining a steady growth trend throughout the culture period. Notably, Treatment F performed as the suboptimal group during the entire culture period, with an average root number of 3.50. Rooting initiated between days 20–25, and the peak period occurred between days 35–55. Most importantly, the high-concentration Treatment I performed the best, with a steady increase in rooting throughout the culture period, achieving an average of 3.50 roots per cutting. Rooting initiated between days 20–25, and the peak period extended from days 25–55, indicating that this hormone combination can promote continuous and stable root development, with high application potential.

In conclusion, Treatment I (0.10 + 2.00 $mg \cdot L^{-1}$) showed the most significant promotion effect on root number of Actinidia arguta stems, demonstrating high application potential.

3.3. Effects of Different Hormone Combinations on Root Length and Growth Trend

Statistical results indicated that different concentration combinations of NAA and 6-BA caused significant differences in root length and overall growth trends of Actinidia arguta stems. Treatment I (NAA 0.10 $mg \cdot L^{-1}$ + 6-BA 2.00 $mg \cdot L^{-1}$) performed the best: average root length in replicate groups increased rapidly from day 25 onwards, showing a significant upward trend, reaching approximately 5.60 cm by day 55, which was significantly higher than other treatment groups. This indicated good root elongation capacity, suggesting that a reasonable ratio of NAA to 6-BA has a positive promoting effect on root length development.

In addition, Treatments H and G showed good performance: average root length in replicate groups increased steadily from days 30–45, reaching 3.10 cm and 1.80 cm by day 55, respectively, reflecting strong root-promoting ability with a more obvious growth rate in the late culture stage. This indicated that appropriate concentrations could effectively stimulate cell elongation and root primordium development. The medium-concentration groups E and F maintained a stable growth trend throughout the culture period, with root length increasing steadily from days 30–45 and reaching 1.20 cm and 0.90 cm by day 55, respectively.

In contrast, the medium-low concentration groups (B, C, D) had an average root length of less than 1.20 cm, with no significant differences between groups. Roots were short and growth was restricted, indicating weak induction effects of low hormone concentrations. Treatment A had an average root length of only 0.08 cm, indicating that cuttings could hardly form effective roots under low-concentration conditions. The CK group showed no effective root length, further confirming the importance of plant hormones for root growth.

In summary, an appropriate combination of NAA and 6-BA concentrations can significantly increase root elongation rate and final root length, showing good root-promoting potential. Treatment

H exhibited stable performance in rooting trend and root length growth, making it a suboptimal hormone ratio for application. The high-concentration Treatment I performed best in all root indicators, particularly with a significant increase in root length, making it the most effective root-promoting treatment in this experiment. This hormone combination has broad application prospects, which is beneficial for improving propagation efficiency and seedling quality of Actinidia arguta.

3.4. Effects of Different Hormone Combinations on Root Diameter and Growth Trend

Statistical analysis showed that different concentration combinations of NAA and 6-BA led to significant differences in root diameter and overall growth trends of Actinidia arguta cuttings. Treatment I (NAA 0.10 mg·L^{-1} + 6-BA 2.00 mg·L^{-1}) was the most prominent: root diameter increased significantly from days 30–55, reaching approximately 1.80 mm by day 55, which was much higher than other treatment groups. This indicated a significant advantage in root thickening, suggesting that the high-concentration hormone combination had the most significant effect on promoting root diameter increase. Thicker roots not only facilitate the absorption of nutrients from the medium but also enhance the mechanical support capacity of stem segments, improving survival rate and stress resistance during transplantation.

In addition, Groups G and H also performed well, with root diameter increasing steadily from days 30–55 and reaching 0.90 mm by day 55, indicating that high-concentration 6-BA combined with an appropriate amount of NAA promotes root thickening. As medium-concentration groups, E and F showed a stable increase in root diameter from days 35–55, reaching 0.80 mm and 0.60 mm by day 55, respectively, demonstrating good regulatory potential.

In contrast, low-concentration groups such as A, C, and D showed slow growth, with root diameter remaining at a low level (average root diameter: 0.05 mm, 0.50 mm, and 0.33 mm, respectively), indicating limited effects of these concentration combinations on inducing root thickening. Additionally, the CK group showed no effective root diameter, confirming the crucial role of plant hormones in regulating root diameter differentiation and growth.

Notably, Treatment B had an average root diameter of 1.10 mm, with the peak growth period between days 35–55, which was significantly higher than other medium-low concentration groups, showing strong effects on promoting root thickening. This may be due to a more balanced hormone ratio in Treatment B or higher adaptability of plants to this concentration combination. This treatment may activate the activity of root meristem even at low concentrations, promoting sufficient expansion of primary tissues and early differentiation of the vascular system, thereby forming thicker roots.

In summary, the hormone concentration combination in Treatment I most significantly increased root diameter in this experiment, thereby improving root robustness and overall quality, which is conducive to achieving rapid propagation of Actinidia arguta while ensuring quality.

3.5. Analysis and Description of Root Growth Status in the Optimal Treatment Group

Experimental results revealed that hormone ratios significantly affected root morphology, vigor, and development in Actinidia arguta cuttings. Treatment I (NAA 0.10 mg·L^{-1} + 6-BA 2.00 mg·L^{-1}) performed optimally, with robust, elongated root systems. Its root bases were pinkish-brown (high maturity), main roots yellowish-green (strong growth), and lateral roots distinctly white (vigorous differentiation). With an average root diameter of 1.80 mm, average length of 5.60 cm, and main roots reaching 6.0–10.5 cm, it formed a dense, functional root system, confirming the hormone combination's superior promotional effect.

Treatment H (0.05 + 2.00 mg·L^{-1}) was suboptimal, with pinkish-brown root bases, yellowish-green main roots, and translucent milky new roots (high activity). It had an average root diameter of 0.90 mm, length of 3.10 cm, and main roots 3.80–8.0 cm, forming a compact structure supporting aboveground growth, with dense lateral roots and active root hairs enhancing absorption.

Treatment G (0.01 + 2.00 mg·L^{-1}) showed good development: pinkish-brown bases, yellowish-white roots (mature transport functions), and milky new roots (high vitality). With 0.90 mm diameter, 1.80 cm length, and main roots 2.5–6.0 cm, it exhibited stable structure and absorption potential, aided by dense root hair villi.

Treatment F (0.10 + 1.50 mg·L^{-1}) had whitish-green roots with pinkish bases, shorter main roots

(1.20–1.80 cm), fewer lateral branches, but abundant root hairs. Though root quantity matched Treatment I, overall performance lagged behind G, H, and I.

In summary, Treatment I was superior in root number, length, and branching. G, H, and F followed, with F useful for prioritizing root quantity. These results support efficient propagation and large-scale cultivation.

4. Conclusion and Discussion

4.1. Optimization Scheme and Research Prospects for Rooting Hormones in Actinidia arguta

Treatment I (NAA 0.10 mg·L^{-1} + 6-BA 2.00 mg·L^{-1}) exhibited the most prominent performance across all indicators: the average rooting rate reached as high as 94.00%, with an average of 3.50 roots per cutting, an average root length of 5.60 cm, and a maximum root length of 10.5 cm. The peak rooting period occurred between days 25–55; average root length increased rapidly from day 25 onwards, and root diameter increased significantly between days 30–55. The root system was well-developed with vigorous differentiation and strong absorption capacity, making it the optimal hormone combination in this experiment.

Although this study preliminarily identified the optimal ratio of NAA to 6-BA as 0.10 mg·L^{-1} + 2.00 mg·L^{-1}, root development regulation is a complex physiological process, and numerous influencing factors require further exploration. This research provides basic theoretical support and technical basis for the cutting propagation of Actinidia arguta. A comparison of the results with previous studies revealed that Meng Yijun et al. [9] screened the optimal formula as 1/2 MS medium supplemented with 0.4 mg·L^{-1} IBA using different basal media and multiple hormone combinations, which significantly improved rooting efficiency. Additionally, Zhao Rui et al. [10] used buds germinated from Actinidia arguta branches as explants and explored in vitro rapid propagation technology by adding different concentrations of auxins (NAA and IBA) to MS basal medium. Their results showed that the optimal rooting medium was 1/2 MS + 0.4 mg·L^{-1} IBA + 30 g·L^{-1} sucrose + 2.0 g·L^{-1} activated carbon, with a rooting rate of 100%, robust roots, abundant quantity, and good rooting quality. Therefore, future studies could introduce combined hormone combinations, integrate dynamic hormone determination, histological section observation, and qRT-PCR analysis to systematically investigate the expression patterns of key root-regulating genes under different hormone concentrations.

Currently, most studies on Actinidia arguta explant culture use apical buds, stem segments, leaves, and axillary buds as explants. To date, there are only a few reports on using reproductive organs such as embryos, endosperms, and anthers as explants in the field of Actinidia arguta tissue culture. Thus, in future research on optimizing and expanding the tissue culture system of Actinidia arguta, exploring reproductive organs (e.g., embryos, endosperms, anthers) as explants could be a specific direction. This would provide better solutions for the rapid propagation and large-scale seedling production of Actinidia arguta and lay a foundation for revealing the mechanism of hormone-regulated root formation.

4.2. Analysis of Differences in Rooting Rate of Actinidia arguta

In plant tissue culture, different plant species and their growth stages have distinct requirements for culture conditions. Multiple factors, such as light intensity and duration, medium pH, temperature, humidity, types and concentrations of plant growth regulators, and material age, affect callus induction, root initiation and development, and subsequent redifferentiation processes to varying degrees [9].

The results of this experiment showed that the high-concentration Treatment I (NAA 0.10 mg·L^{-1} + 6-BA 2.00 mg·L^{-1}) performed excellently in multiple indicators, including rooting rate, root number, root length, and root morphology. However, it is noteworthy that this treatment group had a relatively large standard deviation, indicating certain biological variability among different replicates. This variability may be closely related to differences in the physiological state of cuttings, sampling positions, material age, and minor fluctuations in medium sterilization and operating environment.

To further improve the repeatability and stability of experimental results, future experiments could expand the number of replicates, strictly control explant sources, standardize physiological conditions, optimize aseptic operation procedures, and finely regulate culture conditions to reduce environmental contamination. This would provide more reliable technical support for the standardization and large-scale application of the Actinidia arguta tissue culture system.

4.3. Analysis of Contamination Causes in Tissue Culture and Improvement Methods

During the tissue culture of Actinidia arguta, researchers observed a relatively high contamination rate in Petri dishes, reaching up to 15%. Analysis of potential causes revealed that incomplete sterilization of primary materials was a key factor. Despite using multi-step sterilization methods such as 75% ethanol and 0.1% $HgCl_2$, endogenous contamination may still occur if the cuttings have hidden wounds or attached bacterial spores on their surface. Secondly, after high-temperature and high-pressure sterilization of the medium, exogenous microbial contamination is easily introduced during cooling and dispensing if the aseptic control of the operating environment is inadequate. Additionally, non-standard hand disinfection of operators and repeated cross-contamination during instrument use may also serve as potential contamination sources.

After contamination occurs, common manifestations include white, grayish-green villous or flocculent colonies on the Petri dish walls or medium surface. These colonies can spread rapidly, inhibit normal growth of cuttings, cause tissue browning and necrosis, and severely affect the accuracy and repeatability of experimental data. Therefore, future experiments should further strengthen the standardized procedures for primary material treatment, strictly implement aseptic operation protocols, and, if necessary, introduce plant protectants (e.g., PPM) or antibiotics such as penicillin-streptomycin to assist in contamination control. By optimizing operating procedures and sterilization systems, it is expected to effectively reduce the contamination rate, improve culture success rate, and enhance the stability and repeatability of experiments.

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