

Article

Optimization of an Open Micro-Cutting Propagation Protocol for *Taxus × media*

Chenwei Wu ^{1,†}, Meijing Feng ^{2,†}, Luyuan Jiang ¹, Zhaoyang Meng ¹, Siyu Xia ¹, Menglu Yang ¹, Duanfen Chen ^{3,*} and Yanfang Yang ^{1,*}

¹ State Key Laboratory of Tree Genetics and Breeding, Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China; wcw6636@163.com (C.W.); bachelorjly@163.com (L.J.); mengzhaoyang0815@163.com (Z.M.); xsy322427@163.com (S.X.); yml_1116@163.com (M.Y.)

² Hengshui College of Vocational and Technology, Hengshui 053000, China; fengmj@163.com

³ College of Horticulture, Hebei Agricultural University, Baoding 071001, China

* Correspondence: chenduanfen@163.com (D.C.); echoyyf@caf.ac.cn (Y.Y.)

† These authors contributed equally to this work.

Abstract

This study aimed to establish and optimize an open in-bottle micro-cutting technique for *Taxus × media*. An orthogonal experimental design was adopted. Four core factors were optimized: light regime, plant growth regulator (PGR) type, PGR concentration, and PGR soaking time. Analysis of variance (ANOVA) and Duncan's multiple-range test were performed to analyze the statistical significance of each factor. The optimal combination was identified. Branches were soaked in a 300 mg·L⁻¹ IBA solution for 15 min. They were then cultured under 12 h alternating light/dark conditions in an illumination incubator. Under these conditions, the cutting rooting rate reached a maximum of 45.83%. The optimized open tissue culture system established in this study provides reliable technical support for the clonal propagation of *T. × media*.

Keywords: *Taxus × media*; tissue culture; micro-cutting; orthogonal test

1. Introduction

Paclitaxel (Taxol), a tetracyclic diterpenoid compound, exerts potent anti-tumor activity by inhibiting microtubule bundle depolymerization and microtubule organizing center formation. This mechanism arrests cancer cell mitosis and ultimately triggers tumor cell apoptosis [1–3]. Naturally, paclitaxel is exclusively synthesized in *Taxus* (yew) species. However, its accumulation level is extremely low. Specifically, its content in yew bark is merely 0.01–0.03%. In branches and leaves, it is approximately 0.1–1.67 mg/g. Additionally, *Taxus* trees grow exceptionally slowly [4,5]. The global cancer incidence is rising. Consequently, the international demand for paclitaxel continues to grow annually. Accordingly, rational exploitation, efficient utilization, and germplasm conservation of *Taxus* resources have drawn widespread global attention.

To date, great progress has been made in establishing seed germination and asexual propagation systems for *Taxus* species. Nevertheless, several bottlenecks still restrict their large-scale commercial production. *Taxus* seeds suffer from an overly long germination cycle. Conventional cutting propagation also has drawbacks. It entails high labor input and time-consuming seedling management [5].

Plant tissue culture enables rapid clonal propagation. It has distinct advantages. These include low production costs, easy management, and high explant utilization efficiency.



Academic Editor: Sergio Ruffo Roberto

Received: 17 June 2026

Revised: 28 July 2026

Accepted: 29 July 2026

Published: 3 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

It is also free from geographical and climatic constraints. Tissue culture-based rapid propagation of woody plants has achieved remarkable advances. To date, successful induction of adventitious roots, adventitious buds, and in vitro regenerated plantlets has been documented in multiple *Taxus* species, such as *T. cuspidata*, *T. yunnanensis*, and *T. baccata* [6–9].

Open plant tissue culture is an innovative technique. It employs bacteriostatic agents. These agents eliminate the reliance on stringent aseptic conditions required for conventional tissue culture. This approach allows culture operations to be conducted under natural open environments, eliminating the need for high-pressure sterilization of culture media and equipment, as well as laminar flow cabinets. Compared with traditional tissue culture methods, open tissue culture simplifies operation procedures, cuts production costs, and enhances the growth performance and quality of tissue-cultured seedlings [10,11].

T. × media, a natural interspecific hybrid of *T. cuspidata* and *T. baccata*, accumulates markedly higher paclitaxel concentrations in roots, stems, and leaves than other *Taxus* species. Furthermore, it possesses strong cold resistance and presents a high rooting capacity in conventional cutting propagation [12,13]. In the present study, using *T. × media* as the experimental material, an open cutting rooting and tissue culture system was established to provide technical references for the micropropagation of *Taxus* and other woody forest plants.

2. Materials and Methods

2.1. Plant Materials

Healthy *T. × media* plants were grown in the field in Jinan City, Shandong Province, China. Semi-lignified 1–2-year-old branches were collected from 6-year-old *T. × media* plants in June 2019 for the open micro-cutting experiment. The branches were cut into 3–5 cm segments. Leaves were retained. The segments were placed in a beaker. They were rinsed under running tap water for 1.5 h. The segments were then surface-sterilized with 75% (*v/v*) ethanol for 30 s. They were rinsed 5 times with sterile water. Next, they were immersed in a 0.1% (*w/v*) mercuric chloride (HgCl_2) solution for 6–12 min. The duration was adjusted according to the branch lignification degree. Finally, they were rinsed 5–8 times with sterile water for subsequent use.

2.2. Preparation of Open In-Bottle Cutting Medium

The initial cutting medium comprised 1/2 Murashige and Skoog (MS) basal medium supplemented with $8 \text{ g} \cdot \text{L}^{-1}$ agar. Following an agar concentration optimization experiment conducted within the range of $2.5\text{--}6.0 \text{ g} \cdot \text{L}^{-1}$ (Section 2.5), the optimal concentration was determined to be $4.0\text{--}4.5 \text{ g} \cdot \text{L}^{-1}$. The medium was boiled and adjusted to pH 5.8–6.0. When cooled to approximately 50°C , the corresponding bacteriostatic agent was added, without subsequent high-temperature and high-pressure autoclaving.

2.3. Natural Colony Contamination Test

The medium was prepared in an open environment without autoclaving. When cooled to approximately 50°C , different bacteriostatic agents were added to the non-solidified agar medium. The tested agents included sodium hypochlorite solution, 70% mancozeb wettable powder, 75% chlorothalonil wettable powder, 50% carbendazim wettable powder, and potassium sorbate. For the selected agent, the required amount of commercial formulation was weighed and suspended in a small volume of sterile water with stirring to obtain a homogeneous suspension; the suspension was neither filtered nor autoclaved. Potassium sorbate was set at six concentration gradients: 0.05, 0.1, 0.25, 0.5, 1.0, and $1.5 \text{ g} \cdot \text{L}^{-1}$. After the medium was dispensed into culture bottles and exposed to the open air for 1 h, the

bottles were sealed and incubated at 25 °C. A total of 31 treatments were established, with 15 replicates per treatment. Contamination status was observed after 50 days of incubation, and the contamination rate was calculated. The reported concentrations refer to the commercial formulation rather than the active ingredient.

2.4. Screening of the Optimal Bacteriostatic Agent and Its Concentration

Based on the results of the natural colony contamination test, two bacteriostatic agents with the best antibacterial effect (mancozeb and chlorothalonil) were selected for further optimization. Four concentration gradients were set for each agent: 0.2, 0.4, 0.6, and 0.8 g·L⁻¹ for 75% chlorothalonil; 0.08, 0.1, 0.2, and 0.3 g·L⁻¹ for 70% mancozeb. The sterilized branch segments were inoculated into the medium supplemented with the above fungicides at corresponding concentrations, and the growth status of the cuttings was analyzed after 45 days of culture.

2.5. Optimization of Agar Concentration

Agar powder was added to 1/2 MS liquid medium at 8 concentration gradients: 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 g·L⁻¹. The medium at each concentration was divided into two groups: one group was supplemented with the optimal concentration of the selected bacteriostatic agent, and the other group was without the bacteriostatic agent. The pH of all media was adjusted to 5.8–6.0, and the media were dispensed into clean culture bottles without autoclaving. The solidification status of the medium was observed after cooling for 2 h, with 5 replicates per treatment.

2.6. Optimization of Rooting Conditions via Orthogonal Test

An orthogonal experimental design was adopted to optimize the rooting conditions of the cuttings, with four factors including light regime, PGR type, PGR concentration, and PGR soaking time. An L9(3⁴) orthogonal array with four factors and three levels was used (Table 1). Factor A was PGR type [1-naphthaleneacetic acid (NAA), rooting powder No. 1 (ABT1), and indole-3-butyric acid (IBA)]; Factor B was PGR concentration (50, 100, and 300 mg·L⁻¹); Factor C was PGR soaking time (5, 15, and 30 min); and Factor D was light regime [12 h light/12 h dark alternation (D1), 12 h light/12 h dark alternation with 50% shading (D2), and 24 h complete darkness (D3)]. Each treatment contained 24 cuttings, arranged in 3 replicates, with 4 culture bottles per replicate and 2 cuttings per bottle. The culture bottle served as the experimental unit for statistical analysis. The growth status of the cuttings was observed daily after inoculation, and the cuttings were transferred to fresh medium regularly according to the medium condition. Rooting parameters were calculated after 112 days of culture.

Additionally, three control treatments (CK1–CK3) were included to assess the baseline effects of soaking time and light regime on rooting in the absence of plant growth regulators (PGRs). In these controls, cuttings were soaked in sterile water without any PGR, with only soaking time and light regime matching the corresponding levels in the orthogonal design. CK1: cuttings soaked in sterile water for 5 min and cultured under a D1 light regime (12 h light/12 h dark alternation); CK2: cuttings soaked in sterile water for 15 min and cultured under D2 light regime (12 h light/12 h dark alternation with 50% shading); CK3: cuttings soaked in sterile water for 30 min and cultured under D3 light regime (24 h complete darkness).

Table 1. L9 (3⁴) Orthogonal experimental design.

Treatment No.	A: PGR Type	B: PGR Concentration (mg·L ^{−1})	C: Soaking Time (min)	D: Light Regime	Treatment Combination
1	1 (NAA)	1 (50)	1 (5)	1 (12 h light/12 h dark)	A1B1C1D1
2	1 (NAA)	2 (100)	2 (15)	2 (12 h light/12 h dark with 50% shading)	A1B2C2D2
3	1 (NAA)	3 (300)	3 (30)	3 (24 h complete darkness)	A1B3C3D3
4	2 (ABT1)	1 (50)	2 (15)	3 (24 h complete darkness)	A2B1C2D3
5	2 (ABT1)	2 (100)	3 (30)	1 (12 h light/12 h dark)	A2B2C3D1
6	2 (ABT1)	3 (300)	1 (5)	2 (12 h light/12 h dark with 50% shading)	A2B3C1D2
7	3 (IBA)	1 (50)	3 (30)	2 (12 h light/12 h dark with 50% shading)	A3B1C3D2
8	3 (IBA)	2 (100)	1 (5)	3 (24 h complete darkness)	A3B2C1D3
9	3 (IBA)	3 (300)	2 (15)	1 (12 h light/12 h dark)	A3B3C2D1

2.7. Culture Conditions

All experiments were performed in an open laboratory environment, i.e., inoculation was conducted directly on a laboratory operating table without the use of an ultra-clean bench. Unless otherwise specified, the medium used was sugar-free, supplemented with agar and the optimal bacteriostatic agent, with pH adjusted to 5.8–6.0 before the addition of the bacteriostatic agent. The cultures were incubated in a light incubator with a 12 h photoperiod, 2500 lx light intensity, and a constant temperature of 25 °C. Every 8 days after inoculation, 6 cuttings were randomly selected from each treatment for morphological observation, and the formation of callus and adventitious roots was recorded.

2.8. Data Analysis

Microsoft Excel 2019 was used for raw data collation, calculation, and visualization. SAS 22.0 software was used for ANOVA and Duncan's multiple-range test for significance analysis. The calculation formulas for each index are as follows:

Contamination rate (%) = (number of contaminated explants/total number of inoculated explants) × 100%

Browning rate (%) = (number of browned explants/total number of inoculated explants) × 100%

Survival rate (%) = (number of surviving explants/total number of inoculated explants) × 100%

Rooting rate (%) = (number of rooted explants/total number of inoculated explants) × 100%

Root effect index (REI) = (average root length × average root number)/total number of inoculated cuttings

3. Results

3.1. Determination of Bacteriostatic Agent Type and Effective Concentration

The medium supplemented with different types and concentrations of bacteriostatic agents was exposed to open air for 1 h, sealed, and incubated in an incubator. The contamination rate was counted after 50 days of culture (Table 2, Figure 1). The results showed that the medium supplemented with potassium sorbate or carbendazim had a contamination rate of 100% at all tested concentrations. Sodium hypochlorite exhibited a certain bacteriostatic effect at concentrations ranging from $0.1 \text{ g}\cdot\text{L}^{-1}$ to $1.5 \text{ g}\cdot\text{L}^{-1}$, but the effect was limited, with the lowest contamination rate of 33.3% at the highest tested concentration of $1.5 \text{ g}\cdot\text{L}^{-1}$. In contrast, mancozeb and chlorothalonil achieved a 0% contamination rate at concentrations of $0.1 \text{ g}\cdot\text{L}^{-1}$ and $0.5 \text{ g}\cdot\text{L}^{-1}$, respectively. The bacteriostatic effect of the tested agents was ranked as follows: mancozeb > chlorothalonil > sodium hypochlorite. The effective bacteriostatic concentration ranges were $0.1\text{--}1.5 \text{ g}\cdot\text{L}^{-1}$ for mancozeb and $0.5\text{--}1.5 \text{ g}\cdot\text{L}^{-1}$ for chlorothalonil.

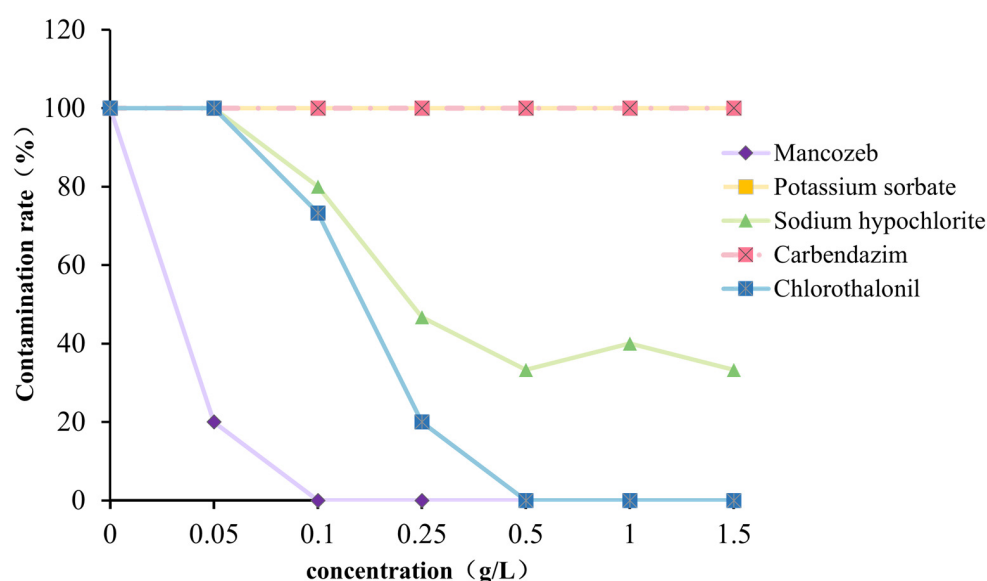


Figure 1. Contamination rate of media supplemented with different concentrations of bacteriostatic agents on the 50th day of culture. Data are mean $\pm$ SE ($n = 15$).

3.2. Determination of the Best Bacteriostatic Species and Concentration

As shown in Tables 3 and 4, the cuttings cultured in the medium supplemented with chlorothalonil had a higher contamination rate and lower survival rate than those treated with mancozeb. For mancozeb treatments, the phytotoxic effect on explants increased with the increase in concentration, accompanied by a gradual rise in browning rate and a significant change in contamination rate. At the mancozeb concentration of $0.1 \text{ g}\cdot\text{L}^{-1}$, the contamination rate was the highest (20.61%), and the survival rate of explants was significantly decreased. In contrast, the highest survival rate of explants (96.67%) was achieved at the mancozeb concentration of $0.08 \text{ g}\cdot\text{L}^{-1}$. Therefore, $0.08 \text{ g}\cdot\text{L}^{-1}$ mancozeb was selected as the optimal bacteriostatic agent for the subsequent experiments of *T. × media* open micro-cutting.

Table 2. Contamination rate of media with different bacteriostatic agents after 50 days of culture.

Concentration (g·L ⁻¹)	Replicate Number	Mancozeb		Potassium Sorbate		Sodium Hypochlorite		Carbendazim		Chlorothalonil	
		Contaminated Count	Contamination Rate (%)	Contaminated Count	Contamination Rate (%)	Contaminated Count	Contamination Rate (%)	Contaminated Count	Contamination Rate (%)	Contaminated Count	Contamination Rate (%)
0	15	15	100.0	15	100.0	15	100.0	15	100.0	15	100.0
0.05	15	3	20.0	15	100.0	15	100.0	15	100.0	15	100.0
0.1	15	0	0.0	15	100.0	12	80.0	15	100.0	11	73.3
0.25	15	0	0.0	15	100.0	7	46.7	15	100.0	3	20.0
0.5	15	0	0.0	15	100.0	5	33.3	15	100.0	0	0.0
1.0	15	0	0.0	15	100.0	6	40.0	15	100.0	0	0.0
1.5	15	0	0.0	15	100.0	5	33.3	15	100.0	0	0.0

Table 3. Effects of mancozeb on the growth of *T. × media* cuttings. Data in the table are mean ± standard error; different lowercase letters in the same column indicate significant differences at the $p < 0.05$ level by Duncan’s multiple-range test.

Bacteriostatic Agent	Concentration (g·L ^{−1})	Browning Rate (%)	Survival Rate (%)	Contamination Rate (%)
Control	0	56.67 ± 17.00 b	50.00 ± 21.60 c	16.67 ± 17.00 b
Mancozeb	0.08	3.33 ± 4.71 c	96.67 ± 4.71 a	6.67 ± 4.71 b
Mancozeb	0.10	20.61 ± 3.74 a	90.30 ± 3.74 ab	16.36 ± 5.14 a
Mancozeb	0.20	0.00 ± 0.00 c	80.00 ± 16.33 bc	40.00 ± 28.33 b
Mancozeb	0.30	0.00 ± 0.00 c	73.33 ± 20.55 bc	26.67 ± 12.47 b

Table 4. Effects of chlorothalonil on the growth of *T. × media* cuttings. Data in the table are mean ± standard error; different lowercase letters in the same column indicate significant differences at the $p < 0.05$ level by Duncan’s multiple-range test.

Bacteriostat	Concentration/(g·L ^{−1})	Brown Rate/%	Survival Rate/%	Contamination Rate/%
Control	0	56.67 ± 17.00 b	50 ± 21.60 c	16.67 ± 17.00 b
Chlorothalonil	0.20	13.33 ± 4.71 b	73.33 ± 9.43 a	10.00 ± 0.00 a
	0.40	50.00 ± 16.33 a	80.00 ± 16.33 a	16.67 ± 4.71 a
	0.60	0.00 ± 0.00 b	76.67 ± 4.71 a	16.67 ± 4.71 a
	0.80	0.00 ± 0.00 b	66.67 ± 17.00 a	10.00 ± 8.16 a

3.3. Optimization of Agar Concentration in the Cutting Medium

As shown in Table 5, the medium with 2.5 g·L^{−1} agar was too soft to fix the cuttings, while the medium with 6.0–8.0 g·L^{−1} agar was excessively hard, which easily caused bending of the cuttings or damage to the basal incision during inoculation. The medium with 4.0–4.5 g·L^{−1} agar exhibited moderate hardness, which could effectively support and fix the cuttings without causing mechanical damage. In addition, there was no significant difference in the solidification degree of the medium with or without mancozeb supplementation, indicating that mancozeb had no effect on the hardness of the agar medium.

Table 5. Solidification degree of medium with different agar concentrations.

No.	Agar Concentration (g·L ^{−1})	Solidification Degree of the Medium
1	2.5	Solidified but highly fluid
2	3.0	Solidified, fluid, adheres to the bottle wall
3	3.5	Solidified, shakeable, can fix explants, adheres to the bottle wall
4	4.0	Moderate hardness, fully solidified, no wall adhesion or shaking, can stably fix explants
5	4.5	Moderately hard
6	5.0	Relatively hard
7	5.5	Relatively hard
8	6.0	Hard

3.4. Optimization of Rooting Conditions via Orthogonal Test

3.4.1. Rooting Performance of Different Treatment Combinations

The rooting performance of *T. × media* cuttings under different orthogonal treatment combinations is shown in Table 6. Treatment 9 (A3B3C2D1) achieved the best comprehensive rooting performance, with the highest rooting rate (45.83%), average root number (4.75), longest adventitious root length (4.43 cm), and root effect index (REI of 1.741). The blank control (CK1) ranked second, with a rooting rate of 33.33%, an average root number

of 3.50, the longest adventitious root length of 4.09 cm, and an REI of 1.260. No rooting was observed in CK2, CK3, Treatment 3, and Treatment 4.

Table 6. Rooting performance of *T. × media* micro-cuttings under different treatments. All data in the table are weighted averages of each treatment.

Treatment No.	Rooting Rate (%)	Average Root Number per Rooted Explant	Longest Adventitious Root Length (cm)	Root Effect Index (REI)
CK1	33.33	3.50	4.09	1.26
CK2	0.00	0.00	0.00	0.00
CK3	0.00	0.00	0.00	0.00
1	25.00	3.12	5.36	1.37
2	12.50	1.00	3.55	0.30
3	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00
5	29.17	4.58	4.34	1.54
6	4.17	2.00	1.55	0.52
7	16.67	1.50	3.32	0.41
8	4.17	1.00	1.33	0.22
9	45.83	4.75	4.43	1.74

3.4.2. Range Analysis of the Orthogonal Test

Range analysis was performed based on the rooting rate of the 9 orthogonal treatment combinations (Table 7, Figure 2). The magnitude of the range R value reflects the importance of each factor on the rooting rate: the larger the R value, the greater the influence of the corresponding factor. The results showed that the influence of the four tested factors on the rooting rate of *T. × media* cuttings was ranked as follows: light regime > PGR type > PGR soaking time > PGR concentration. The light regime was the dominant factor affecting the rooting rate. Among all treatment combinations, A3B3C2D1 achieved the highest rooting rate (45.83%), followed by A2B2C3D1 (29.17%), while no rooting was observed in A1B3C3D3 and A2B1C2D3. The optimal level combination for each factor was A3 (IBA), B3 (300 mg·L^{−1}), C2 (15 min), and D1 (12 h light/12 h dark alternation), which was consistent with the best-performing Treatment 9.



Figure 2. Adventitious root formation of *T. × media* cuttings under optimal treatment (A3B3C2D1: 300 mg·L^{−1} IBA, 15 min soaking, 12 h light/12 h dark alternation): (a) rooting status at 70 days of culture; (b) rooting status at 112 days of culture.

Table 7. Range analysis of the L9(3⁴) orthogonal test for rooting rate of *T. × media* cuttings. *K* is the sum of rooting rates at each level of the corresponding factor; *k* is the average value of *K*; and *R* is the range value.

Treatment No.	A: PGR Type	B: PGR Concentration	C: Soaking Time	D: Light Regime	Treatment Combination	Rooting Rate (%)
1	1	1	1	1	A1B1C1D1	25.00
2	1	2	2	2	A1B2C2D2	12.50
3	1	3	3	3	A1B3C3D3	0.00
4	2	1	2	3	A2B1C2D3	0.00
5	2	2	3	1	A2B2C3D1	29.17
6	2	3	1	2	A2B3C1D2	4.17
7	3	1	3	2	A3B1C3D2	16.67
8	3	2	1	3	A3B2C1D3	4.17
9	3	3	2	1	A3B3C2D1	45.83
K1	37.50	41.67	33.34	100.00		
K2	33.34	45.84	58.33	33.34		
K3	66.67	50.00	45.84	4.17		
k1	12.50	13.89	11.11	33.33		
k2	11.11	15.28	19.44	11.11		
k3	22.22	16.67	15.28	1.39		
R	11.11	2.78	8.33	31.94		

3.4.3. Variance Analysis and Multiple Comparisons

ANOVA was performed to verify the significance of each factor on the rooting rate (Table 8). The results showed that light regime ($p < 0.001$) and PGR type ($p < 0.05$) had extremely significant and significant effects on the rooting rate of '*T. × media*' cuttings, respectively. In contrast, PGR concentration and soaking time had no significant effect on rooting rate ($p > 0.05$).

Table 8. ANOVA of the orthogonal test for rooting rate.

Source	Sum of Squares	df	Mean Square	F Value	p Value
Corrected model	3781.034	8	472.629	9.426	0.001
Intercept	4726.188	1	4726.188	94.256	<0.001
PGR type (A)	702.404	2	351.202	7.004	0.015
PGR concentration (B)	7.710	2	3.855	0.077	0.927
Soaking time (C)	146.627	2	73.313	1.462	0.282
Light regime (D)	2924.293	2	1462.147	29.160	<0.001
Error	451.278	9	50.142		
Total	8958.500	18			
Corrected total	4232.312	17			

Duncan's multiple-range test results (Table 9) showed that the differences in rooting rate among different levels of each factor did not reach a significant level ($p > 0.05$), indicating that the variation among levels might be attributed to random error. However, the *p*-value for the light regime factor (0.138) was relatively small, and the rooting rate under the D1 level (12 h light/12 h dark alternation) was substantially higher (33.33%) than that under D2 (11.11%) and D3 (1.39%), suggesting that light regime might be a key factor affecting rooting rate and warrants further optimization in future studies.

Table 9. Duncan’s multiple-range test for the rooting rate of each factor.

PGR Type	Mean Rooting Rate	PGR Concentration	Mean Rooting Rate	Soaking Time	Mean Rooting Rate	Illumination	Mean Rooting Rate
A1	11.11 b	B1	15.28 a	C1	12.50 a	D1	33.33 c
A2	25.00 a	B2	16.67 a	C2	19.44 a	D2	12.50 b
A3	12.50 b	B3	16.67 a	C3	16.67 a	D3	2.78 a

Note: PGR: plant growth regulator. A1–A3: PGR type: A1 = NAA; A2 = ABT1; A3 = IBA. B1–B3: PGR concentration: B1 = 50 mg·L^{−1}; B2 = 100 mg·L^{−1}; B3 = 300 mg·L^{−1}. C1–C3: soaking time: C1 = 5 min; C2 = 15 min; C3 = 30 min. D1–D3: light regime: D1 = 12 h light/12 h dark; D2 = 12 h light/12 h dark with 50% shading; D3 = 24 h complete darkness. Values followed by different lowercase letters within the same factor column differ significantly at $p < 0.05$ according to Duncan’s multiple-range test.

4. Discussion

Taxus species are rare medicinal gymnosperms, and paclitaxel extracted from yew bark possesses prominent antitumor activity against multiple malignant tumors [2]. Seed propagation and cutting propagation are the main reproduction methods for *Taxus*, and cutting propagation has become the dominant commercial breeding mode of *T. × media* [13]. Conventional propagation methods have long cycles and low efficiency. In contrast, tissue culture-based micropropagation has unique advantages. These include a high propagation coefficient, controllable culture conditions, and low explant material consumption. It also supports the production of virus-free seedlings and new variety breeding [10,11]. In recent years, key techniques of *Taxus* tissue culture have been greatly improved, involving light, temperature, and pH regulation; explant selection; and hormone combination optimization [7,14–16].

Open tissue culture relies on bacteriostatic agents to replace rigorous aseptic environments, which eliminates autoclaving and ultraclean bench operation and effectively reduces production costs. The selection of bacteriostatic agents is the core prerequisite for successful open tissue culture, whereas universal bacteriostatic agents with stable effects are difficult to screen for different plant species [11,17–19]. The optimal agent type, concentration, and combination vary greatly among plants. For example, mancozeb and carbendazim are suitable for open culture of *Amygdalus pedunculatus* [20]. Meanwhile, chlorothalonil at an appropriate concentration (0.2 g·L^{−1}) exhibited excellent bacteriostatic effects on *Acacia* species explants without obvious growth inhibition [21,22]. Plant-derived bacteriostatic substances have good histocompatibility but unstable effects and high costs, which restrict their large-scale application [23]. In the present study, five common broad-spectrum bacteriostatic agents were screened, and 0.08 g·L^{−1} mancozeb presented the best comprehensive performance with the lowest contamination rate and the highest explant survival rate of 96.67%. In contrast, carbendazim, potassium sorbate, and sodium hypochlorite showed poor bacteriostatic effects. Different responses to bacteriostatic treatments fully reflect plant species specificity, indicating that targeted screening must be carried out for different materials. Additionally, this study simultaneously completed the agar concentration gradient screening of the culture medium, determining 4.0–4.5 g·L^{−1} agar as the optimal ratio, which resolved the practical difficulties of the substrate being too soft to fix cuttings or too hard to damage the basal wounds of cuttings, thereby constructing a sugar-free simple cutting culture medium with stable and controllable composition.

Light regime is a critical factor affecting adventitious root formation in cuttings. Light regulates photosynthesis, carbohydrate accumulation, and endogenous hormone synthesis, thereby directly influencing rooting [24]. Excessively strong light leads to leaf dehydration and branch burning, while weak light causes leaf yellowing and abscission [25]. Appropriate light alternation is therefore essential for cutting rooting. In this study, orthogonal

analysis confirmed that the light regime was the primary factor affecting the rooting rate. The 12 h light/12 h dark alternating culture significantly improved rooting performance.

Among the many factors affecting adventitious root formation in cuttings, in addition to the light regime as the dominant factor, the type, concentration, and treatment duration of PGRs also play a crucial regulatory role [26,27]. Previous studies have shown that soaking cuttings with $200 \text{ mg} \cdot \text{L}^{-1}$ ABT for 30 min is conducive to rooting of *T. × media*, achieving a rooting rate of 73.61% [28]; rapid immersion with $1000 \text{ mg} \cdot \text{L}^{-1}$ NAA for 5 s can yield a rooting rate of 98.4% [29]. Notably, these studies were all focused on conventional field cutting propagation, with considerable variations in hormone type, concentration, and treatment method. In the present experiment, the orthogonal analysis confirmed that PGR type was the second most important factor after light regime, and IBA (indole-3-butyric acid) showed significantly better rooting-promoting effects than NAA (1-naphthaleneacetic acid) and ABT1 rooting powder in *T. × media*. With $300 \text{ mg} \cdot \text{L}^{-1}$ IBA soaking treatment for 15 min, the maximum rooting rate reached 45.83%, which was significantly higher than the 27.78% rooting rate reported in earlier conventional tissue culture [30]. However, the rooting rate (45.83%) remains lower than the 70–90% typically achieved by conventional field cutting propagation of *T. × media* [28,29,31]. The possible reasons were complex, and further research will focus on the effects of genotype, explant type, and culture conditions.

Most existing studies on open tissue culture of *Taxus* species have focused solely on single-factor screening of bacteriostatic agents or optimization of exogenous hormone ratios, lacking systematic and synergistic investigation of multiple factors. This represents the core methodological innovation of the present study. This study employed an $\text{L9}(3^4)$ orthogonal experimental design to simultaneously optimize four critical cultivation factors, namely bacteriostatic agents, culture medium substrate, light regime, and exogenous hormones. ANOVA and Duncan's multiple-range test further clarified the significant main effects of these factors on rooting. Interaction effects were not analyzed due to the limitations of the $\text{L9}(3^4)$ orthogonal design. For the first time, a complete and integrated open in vitro micro-cutting propagation system for *T. × media* was established. This system differs from previous single-variable experiments and achieves a breakthrough in low-cost micro-cutting propagation technology for woody medicinal plants. Nevertheless, this system was currently validated only in *T. × media*, and its applicability to other *Taxus* species remains to be further verified.

Limitations of the Study

Several limitations of this study should be acknowledged. First, the surface sterilization procedure relied on mercuric chloride (HgCl_2), which is highly toxic and environmentally hazardous; alternative sterilization methods should be explored in future studies. Second, although mancozeb showed promising bacteriostatic effects, its potential phytotoxicity during long-term culture requires further investigation. Third, acclimatization and transplantation of rooted cuttings to soil were not performed, which is essential for practical application. Fourth, the study was conducted using a single genotype of *T. × media*, and the applicability to other genotypes or *Taxus* species remains to be validated. Finally, the rooting rate of 45.83%, while higher than some tissue culture reports, remains lower than the 70–90% typically achieved by conventional field cutting propagation. The relatively modest improvement over the control (12.5 percentage points) further indicates that further optimization of the open micro-cutting system is warranted to enhance practical efficiency, including explant preparation and culture management.

In summary, the open micro-cutting system established in this study can effectively reduce culture costs, control contamination, and improve cutting survival and rooting performance. It provides theoretical reference and technical support for the popularization

of open tissue culture and industrialized seedling cultivation of *Taxus* and other woody plants. Future research should focus on the development of compound bacteriostatic agents, long-term subculture adaptation, and synchronous seedling domestication so as to further promote the practical application of open tissue culture technology.

5. Conclusions

In this study, an open in-bottle micro-cutting rooting system was successfully established for *T. × media*. The cutting medium was simplified to consist of 1/2 MS basal medium supplemented with 4.0 g·L^{−1} agar and 0.08 g·L^{−1} mancozeb, enabling the cuttings to grow in a composition-controllable matrix without the need for autoclaving. The optimal rooting treatment combination was identified as A3B3C2D1: semi-lignified branches of *T. × media* were soaked in 300 mg·L^{−1} IBA for 15 min. They were then cultivated under a 12 h light/12 h dark alternation regime. The light intensity was 2500 lx. Under these optimized conditions, the highest rooting rate of 45.83% was achieved. All operations were conducted in an open laboratory environment, eliminating the requirement for an ultra-clean bench and autoclaving of inoculation equipment, which significantly reduces the production cost of *T. × media* culture propagation. Collectively, this study provides a solid theoretical foundation and reliable technical reference for the research and practical application of open in-bottle micro-cutting technology in *T. × media*. Further studies are needed to validate the applicability of this protocol to other *Taxus* species.

Author Contributions: Conceptualization, Y.Y. and D.C.; methodology and data analysis, M.F. and L.J.; writing—original draft preparation, C.W. and Y.Y.; writing—review and editing, Z.M., M.Y. and S.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of China (Grant No. 32371907) and the Fundamental Research Funds of the State Key Laboratory of Tree Genetics and Breeding (Chinese Academy of Forestry) (Grant No. TGBFRF202511).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Jijie, A.; Iliescu, D.; Sbârcea, L.; Boru, C.; Pătraşcu, D.; Iftode, O.A.; Minda, I.; Trandafirescu, C.; Dehelean, C.A.; Moacă, E. A deep dive into the botanical and medicinal heritage of *Taxus*. *Plants* **2025**, *14*, 1439. [[CrossRef](#)] [[PubMed](#)]
2. Li, Y.P.; Lin, R.; Mu, R.H. Research progress of paclitaxel and its combination therapy against tumor. *J. Med. Coll. Jilin Univ.* **2021**, *42*, 440–442.
3. Zhang, Y.; Xia, M. Application research of *Taxus* extract in daily chemical products. *Green Sci. Technol.* **2021**, *23*, 128–130.
4. Gao, X.; Zhang, N.; Xie, W. Advancements in the cultivation, active components, and pharmacological activities of *Taxus mairei*. *Molecules* **2024**, *29*, 1128. [[CrossRef](#)] [[PubMed](#)]
5. Pan, X.H. Optimization of tissue culture rapid propagation system of *Taxus* and analysis of alkaloid content. *Spec. Econ. Anim. Plants* **2025**, *28*, 46–48.
6. Abbasin, Z.; Zamani, S.; Movahedi, S.; Khaksar, G.; Sayed Tabatabaei, B.E. In vitro micropropagation of yew (*Taxus baccata*) and production of plantlets. *Biotechnology* **2010**, *9*, 48–54.
7. Jiao, J.; Liu, J.; Fu, Y.J.; Wang, X.; Gai, Q.Y.; Lu, Y.; Xu, X.J.; Wang, Z.Y. Study on tissue culture and breeding technology of axillary bud initiation of stem segments of three *Taxus* species. *Bull. Bot. Res.* **2021**, *41*, 721–728.
8. Yang, L.; Niu, Z.L.; Chen, H.G. Study on tissue culture conditions of *Taxus yunnanensis*. *Mod. Agric. Sci. Technol.* **2012**, *11*, 143–144.
9. Yin, J.T.; Su, S.J.; Zhang, Y.D.; Feng, L.N. Study on cutting propagation technology of *Taxus yunnanensis*. *For. Sci. Technol. Commun.* **2017**, *10*, 37–39.
10. Fan, Y.Y.; Xiao, H.J. Development of plant tissue culture and prospect of new technologies. *J. Beijing Vocat. Coll. Agric.* **2024**, *38*, 82–89.

11. Zhou, Y.H. A new method of plant tissue culture—Research overview of open tissue culture. *Chin. Hortic. Abstr.* **2018**, *34*, 57–59.
12. Chen, Y.S. *Chinese Woody Plant Seeds*; China Forestry Publishing House: Beijing, China, 2000; pp. 130–134.
13. Yang, L.Y.; Yang, J.C.; Liu, Y.; Zhang, W. Study on cutting propagation and afforestation of *Taxus media*. *China For. Prod. Spec.* **2022**, *1*, 27–29.
14. Liu, L.L.; Hu, X.Y.; Wang, S.J.; Wang, Z.Y.; Zhang, J.X. Effects of NAA and BA on bud induction and differentiation of *Taxus cuspidata*. In Proceedings of the 2012 Annual Academic Conference of the Chinese Society for Horticultural Science, Yangling, China, 20–21 October 2012.
15. Zhang, J.X.; Li, J.X.; Hu, X.Y.; Wang, Z.Y.; Huang, M.X.; Wang, S.J. Application of mancozeb in open tissue culture of *Taxus cuspidata*. In Proceedings of the 2012 Annual Academic Conference of the Ornamental Horticulture Professional Committee of the Chinese Society for Horticultural Science, Guangzhou, China, 16–18 August 2012.
16. Wang, S.J.; Zhao, Z.R.; Meng, H.W.; Li, X.H.; Zhang, Y.J.; Zhou, Y.J. Effects of light culture conditions on tissue culture efficiency of *Taxus cuspidata* explants. *J. Jilin Univ. Eng. Technol. Ed.* **2021**, *51*, 2313–2318.
17. Jiao, Z.H.; Tang, W.; Liu, Y.H.; Guo, K.; Zhan, S.L.; Peng, D.L. Current status of tissue culture of economic plants and application of new technologies. *Biotechnol. World* **2013**, *5*, 41–42.
18. Cao, G.L.; Li, W.J.; Zhu, M.Q.; Zhang, Y.L.; Zhou, R.; Sun, Y.L. Application of potassium sorbate, sodium benzoate and sodium diacetate in controlling fungal contamination in tissue culture of *Alternanthera bettzickiana*. *For. Sci. Technol. Commun.* **2023**, *6*, 26–29.
19. Han, Y.; Yang, X.Y.; Wu, H.; Xie, S.Z. Screening of bacteriostatic agents for open tissue culture of *Hosta plantaginea*. *Shaanxi J. Agric. Sci.* **2025**, *71*, 15–19.
20. Sun, Z.Y.; Cao, B.; Li, H.F.; Guo, C.H. Experimental study on open tissue culture of *Amygdalus pedunculata*. *Mod. Agric. Sci. Technol.* **2013**, *20*, 66–69.
21. Lu, Y.P.; Chen, Y.J.; Li, M.; Huang, L.J. Establishment of open tissue culture system for *Acacia mangium*. *J. Northwest A F Univ.* **2023**, *51*, 107–114.
22. Huang, L.; Wang, H.; Shahid, M.Q.; Zeng, B. Chlorothalonil: An effective bacteriostatic agent for bud induction of *Acacia auriculiformis* under open condition (non-axenic). *Plant Methods* **2019**, *15*, 5. [[CrossRef](#)] [[PubMed](#)]
23. Huang, M.; Liang, C.H.; Qiu, X.M. Research overview of plant open tissue culture technology. *Hortic. Seed* **2020**, *40*, 26–28.
24. Jiang, H.Z.; Ding, J.P.; Yang, J.L.; Zhu, M.L.; Liu, N.G.; Liang, H.L. Research progress on factors affecting full-light spray cutting of tea plant (*Camellia sinensis*). *China Tea* **2023**, *45*, 19–25.
25. Zhao, X.M.; Huo, C.F.; Shen, H.L. Review on internal and environmental factors affecting rooting of forest tree cuttings. *World For. Res.* **2007**, *20*, 12–16.
26. Huang, C.E.; Teng, Y.F.; Wu, N.N.; Su, F.R. Effects of plant growth regulators on softwood cutting rooting of *Weigela florida* ‘Variegata’. *Northwest Hortic.* **2025**, *12*, 61–63.
27. Chen, Y.Q.; Wei, Y.H.; Wei, B.B.; Pan, H. Effects of plant growth regulators on leaf cutting propagation of four *Sedum* species. *Fujian J. Agric. Sci.* **2018**, *33*, 799–805.
28. Luo, Y.L.; Ye, J.R.; Luo, G.F.; Zhao, G.Q.; Wang, C.H.; Jiang, L.M. Preliminary report on container seedling cultivation of *Taxus media*. *J. Agric.* **2014**, *4*, 57–59, 68.
29. Ma, J. Study on Rapid Propagation Technology System of *Taxus Media* cv. *Hicksi*. Master’s Thesis, Sichuan Agricultural University, Chengdu, China, 2007.
30. Ma, J.; Ma, M.D. Tissue culture and rapid propagation technology of *Taxus media*. *Sci. Silvae Sin.* **2007**, *43*, 30–34.
31. Wang, J.J.; Zhou, D.; Song, X.Y. Analysis of factors affecting cutting survival rate of *Taxus media*. *J. Zhejiang For. Coll.* **2004**, *21*, 357–360.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.