Rapid Propagation Technology for Cyclocarya paliurus (Postprint)

Authors: Huang Ningzhen, Su Jiang, Xian Kanghua, Fu Chuanming, Gong Qingfang, Huang Huijin, He Jinxiang

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Abstract

Cyclocarya paliurus is a fast-growing arboreal species belonging to the monotypic genus Cyclocarya in the family Juglandaceae, endemic to China. It possesses significant medicinal value and is acclaimed as "the third tree in the medical field." However, its seedling propagation is characterized by considerable difficulty and low efficiency. Therefore, this study employed young stem segments of C. paliurus as explants to investigate rapid propagation technology for its seedlings, thereby establishing a foundation for large-scale propagation of superior clones. The results indicated that the optimal sampling period for C. paliurus is from April to June, with slightly lignified stem segments serving as the optimal explants. The most effective surface sterilization method for explants involved immersion in 0.1% HgCl2 for 5–7 min, yielding a sterilization success rate of 54.1% and a survival rate of sterile explants of 88.7%. The primary bud induction medium consisted of MS + 6-BA 2.0 mg L-1 + IBA 0.2 mg L-1 + sucrose 30.0 g L-1, achieving a bud induction rate of 80.5%; after 21 days of culture, the average height of primary buds reached 3.0 cm. The optimal subculture proliferation medium was MS + 6-BA 0.5 mg L-1 + IBA 0.05 mg L-1 + TIBA 0.02 mg L-1 + sucrose 30.0 g L-1; following 35 days of culture, the proliferation coefficient was 7.0 per 35 days, with an average seedling height of 4.5 cm, producing robust buds without vitrification. The seedling strengthening medium prior to rooting comprised MS + 6-BA 0.5 mg L-1 + IBA 0.05 mg L-1 + sucrose 30.0 g L-1; after 35 days of culture, the developed buds were tall and robust, with an average seedling height of 6.0 cm. The rooting medium was 1/2WPM + IBA 1.5 mg L-1 + 5-NGS 4.5 mg L-1 + sucrose 20.0 g L-1; after 40 days of culture, the maximum rooting rate attained 83.3%. The most suitable transplanting substrate for rooted seedlings was peat soil, with the preferable transplanting seasons being March to May and October to November. Following transplanting, seedlings were cultivated for 40 days in a greenhouse with 70% shading, achieving a transplant survival rate of 54.3%–65.6%.

Full Text

Rapid Propagation Technology of *Cyclocarya paliurus*

HUANG Ningzhen, SU Jiang, XIAN Kanghua, FU Chuanming, GONG Qingfang, HUANG Huijin, HE Jinxiang*

Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, Guangxi, China

Abstract

Cyclocarya paliurus is a unique fast-growing tree species in China and the sole member of the genus *Cyclocarya* in the family Juglandaceae. Valued for its important medicinal properties and honored as "the third tree in the medical field," this species faces significant challenges in seedling propagation due to difficult and inefficient breeding methods. To address this, we investigated rapid propagation technology using young stem segments as explants to establish a foundation for large-scale breeding of superior clones. Our results demonstrate that the optimal sampling period is April to June, with slightly lignified stem segments serving as the best explants. The most effective surface disinfection method involves soaking explants in 0.1% HgCl for 5–7 minutes, achieving a disinfection success rate of 54.1% and a sterile explant survival rate of 88.7%. The optimal primary bud induction medium is MS + 6-BA 2.0 mg · L ^{1} + IBA 0.2 mg · L ^{1} + sucrose 30.0 g · L ^{1}, yielding a bud induction rate of 80.5% and an average primary shoot height of 3.0 cm after 21 days. The best subculture proliferation medium is MS + 6-BA 0.5 mg · L ^{1} + IBA 0.05 mg · L ^{1} + TIBA 0.02 mg · L ^{1} + sucrose 30.0 g · L ^{1}, which produces a proliferation coefficient of 7.0 per 35 days, average shoot height of 4.5 cm, and robust, vitrification-free shoots. For shoot strengthening before rooting, the medium MS + 6-BA 0.5 mg · L ^{1} + IBA 0.05 mg · L ^{1} + sucrose 30.0 g · L ^{1} produces tall, robust shoots averaging 6.0 cm after 35 days. The optimal rooting medium is 1/2WPM + IBA 1.5 mg · L ^{1} + 5-NGS 4.5 mg · L ^{1} + sucrose 20.0 g · L ^{1}, achieving a maximum rooting rate of 83.3% after 40 days. The most suitable transplanting substrate is peat soil, with optimal transplanting seasons being March to May and October to November. When cultivated for 40 days in a greenhouse with 70% shading, transplant survival rates range from 54.3% to 65.6%.

Keywords: *Cyclocarya paliurus*, young stem segments, rapid propagation

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Introduction

Cyclocarya paliurus (Batal.) Iljinskaja, belonging to the family Juglandaceae, is a monotypic genus endemic to China. Known as the "money tree" or "palm willow," this rare species survived the Quaternary glaciation and is listed as a

nationally protected endangered plant. Often referred to as the "giant panda of the plant kingdom" and "the third tree in the medical field," its bark and leaves have been documented in traditional medicine for clearing heat, detoxifying, and relieving pain, particularly in treating stubborn dermatitis. For approximately 200 years, local communities have used its leaves to produce health tea (Chinese Herbal Medicine Company, 1994; Fu et al., 2017). Recent research has revealed that *C. paliurus* is rich in polysaccharides, flavonoids, oleanolic acid, terpenoids, organic acids, alkaloids, and sterols (Li et al., 2008; Li J et al., 2008; Tang et al., 2017), exhibiting hypoglycemic, hypotensive, hypolipidemic, immunomodulatory, antioxidant, anti-aging, and anti-tumor effects (Tang et al., 2017; Zou et al., 2018; Han et al., 2009). Currently, health tea products derived from *C. paliurus* have gained recognition not only among domestic consumers but also from the U.S. Food and Drug Administration, Japan' s Ministry of Health, and Germany' s Federal Ministry of Health, representing the first Chinese health tea product certified by the U.S. FDA (Xie and Xie, 2008).

Existing *C. paliurus* resources are scarce and scattered across remote mountainous forests and nature reserves, severely limiting research, development, and application. Moreover, the seeds possess both hard seed coats and deep dormancy characteristics, with severe abortion rates reaching approximately 90% empty shells. Germination typically requires more than a year after sowing, with natural germination rates as low as 0.1–0.5% (Xu and Song, 2004). Additionally, based on our experience and published literature on cutting propagation, *C. paliurus* cuttings generally exhibit difficult rooting and low success rates (Li et al., 2014; He, 2016). Although some studies have reported relatively high rooting rates, the resulting roots are fleshy and transplant survival remains extremely low, preventing widespread application in forestry production (Tong et al., 2016; Yao and Zeng, 2017).

While limited reports on tissue culture and rapid propagation of *C. paliurus* exist (Shangguan et al., 2006; Xie et al., 2009, 2011, 2012; Lu et al., 2013; Wang et al., 2016; Fu et al., 2017; Zhang et al., 2018), they have achieved promising results and valuable insights. For instance, Wang (2016) demonstrated that high-concentration hormone media are unnecessary for primary induction and subculture proliferation of *C. paliurus* stem segments, providing important reference data. However, these studies suffer from small experimental scales, suboptimal subculture material quality, and low rooting rates. To address these issues, we systematically investigated the complete tissue culture and rapid propagation process using young stem segments as explants, providing a reference for further improvement of *C. paliurus* seedling micropropagation technology.

Materials and Methods

1.1 Plant Materials and Disinfection Methods Plant Materials: Experimental *C. paliurus* germplasm originated from Yufeng Mountain, Hubei

Province. Sampled plants were 2–3-year-old specimens cultivated at the Guangxi Institute of Botany. Collections were made during April–June and August–October each year, targeting young branches bearing 5–6 axillary buds.

Explant Processing and Disinfection: Collected branches were defoliated and soaked in 0.1–0.2% detergent solution for approximately 5 minutes, then rinsed thoroughly with tap water. Under aseptic conditions, materials were cut into 3–5 cm segments containing one axillary bud each. Non-lignified (apical 1–2 buds) and slightly lignified (3rd–5th buds below the apex) materials were sorted separately for disinfection. The disinfection protocol involved: (1) 30-second immersion in 75% ethanol, (2) 5–7 minute or 8–10 minute immersion in 0.1% HgCl , (3) 4–5 rinses with sterile water, and (4) placement in sterile stainless steel trays where both ends were trimmed to retain 2–4 cm axillary bud-containing segments for inoculation onto primary induction medium. After 14 days, contamination and survival rates were recorded . Contamination rate was calculated as (contaminated explants/total explants) $\times$ 100%, while survival rate was calculated as (surviving sterile explants/(total explants $-$ contaminated explants)) $\times$ 100%.

1.2 Medium Preparation and Screening Primary Culture Medium: MS and WPM basal media were used as foundations. Based on preliminary experiments, 6-BA (1.0, 2.0, 4.0, 8.0 mg $\cdot$ L ^{1}) and IBA (0.1, 0.2, 0.4, 0.8 mg $\cdot$ L ^{1}) were added at a 10:1 ratio, supplemented with sucrose 30 g $\cdot$ L ^{1} and agar 3.3 g $\cdot$ L ^{1}, pH 5.8 . Optimal primary induction medium was selected by observing and recording bud induction rates and shoot growth. Bud induction rate was calculated as (germinated explants/successfully disinfected explants) $\times$ 100%.

Subculture Proliferation Medium: MS basal medium was supplemented with various concentrations and combinations of plant growth regulators including 6-BA, KT, ZT, IBA, NAA, IAA, and TIBA (triiodobenzoic acid) [TABLE:3 and TABLE:4], with sucrose 30 g $\cdot$ L ^{1} and agar 3.3 g $\cdot$ L ^{1}, pH 5.8. Media formulations were screened for high proliferation coefficients, robust subculture shoots, and stable performance.

Rooting Medium: 1/2MS and 1/2WPM basal media were supplemented with various types, concentrations, and combinations of IBA, NAA, IAA, DA-6 (diethyl aminoethyl hexanoate, also known as amine fresh ester), and 5-NGS (5-nitroguaiacol sodium salt), with sucrose 20 g $\cdot$ L ^{1} and agar 3.3 g $\cdot$ L ^{1}, pH 5.8 [TABLE:5 and TABLE:6]. Appropriate rooting medium formulations were selected.

Each treatment comprised 10–60 replicates: one explant per bottle for primary culture, and 8–10 explants per bottle for subculture and rooting. All experiments were repeated three times. Media were sterilized at 125 °C for 25 minutes before use.

1.3 Inoculation and Culture **Subculture Inoculation:** When subculture shoots reached 4–7 cm, regenerated buds were separated into single buds, defoliated, and cut into single-node segments containing one axillary bud. These were inoculated onto subculture medium at 8–10 inoculation points per bottle for 30–40 days.

Rooting Inoculation: Subculture shoots exceeding 4.5 cm in height with robust growth were selected, excised at the base, and transferred intact to rooting medium at 8–10 plants per bottle for 30–40 days.

Culture Conditions: Temperature (28 ± 3) °C, photoperiod 10–12 $h \cdot d^{-1}$, light intensity 30–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

1.4 Rooting Seedling Hardening and Transplanting When tissue-cultured seedlings developed 2–5 roots exceeding 2.0 cm in length, rooted plantlets were acclimatized with open caps for 3–5 days. After washing away residual medium, roots were disinfected in 1,000× "Special Effect Fungicide for Wilt and Root Rot—Hymexazol · Pyrimethanil · Phenazine" solution for 2–3 minutes. The same solution was sprayed on transplanting substrates (garden soil, peat soil, and coconut fiber) before planting in nursery cups. Seedlings were cultivated in a greenhouse with 70% shading, maintaining ambient and substrate humidity while spraying the fungicide weekly for disease prevention. Transplant survival rates were recorded after 40 days to determine optimal substrate and timing.

1.5 Statistical Analysis Primary culture and disinfection experiments used 60 bottles per treatment with three replicates; subculture and rooting experiments used 10 bottles per treatment with three replicates. All data were analyzed using SPSS statistical software.

Results

2.1 Explant Collection and Disinfection Effects of sampling time, explant type, and disinfection duration on disinfection success and survival rates are summarized in . Results revealed: (1) For August–October collections, contamination rates were high (67.6%) regardless of explant type (apical buds, non-lignified or slightly lignified segments) or disinfection duration, with all explants subsequently browning and dying. (2) For April–June collections, apical buds and non-lignified stem segments completely browned and died within 2–3 days post-disinfection. In contrast, slightly lignified stem segments disinfected for 5–7 minutes showed 45.9% contamination but 88.7% survival, while 8–10 minute disinfection yielded 43.9% contamination with increased browning mortality and reduced survival (65.3%). Statistical analysis indicated no significant difference in contamination rates between 5–7 and 8–10 minute treatments, but survival rates differed significantly.

In summary, optimal explant collection occurs in April–June, with slightly lignified stem segments as the best explant type and 5–7 minute disinfection in 0.1% HgCl as the most effective protocol.

2.2 Primary Culture Slightly lignified stem segments were inoculated onto various primary induction media . After three weeks, growth occurred on both MS and WPM basal media, but MS yielded superior bud induction rates and shoot heights. Low concentrations of 6-BA and IBA (1.0–2.0 mg · L ^{1} + 0.1–0.2 mg · L ^{1}) promoted faster shoot growth, while high concentrations (8.0 mg · L ^{1} + 0.8 mg · L ^{1}) significantly inhibited axillary bud germination and shoot elongation. Overall, *C. paliurus* tolerated a relatively wide range of plant growth regulator concentrations during primary culture, with bud induction rates exceeding 40.0% across 6-BA 1.0–4.0 mg · L ^{1} + IBA 0.1–0.4 mg · L ^{1}. The optimal formulation was MS + 6-BA 2.0 mg · L ^{1} + IBA 0.2 mg · L ^{1}, achieving maximum bud induction rate (80.5%) and shoot height (3.5 cm) [TABLE:2, FIGURE:1-A], making it suitable as the primary induction medium.

2.3.1 Preliminary Subculture Proliferation Based on primary culture results, media formulations in were tested. Findings indicated: (1) *C. paliurus* grew well on media containing 6-BA + IBA or 6-BA + IAA, but the former was superior for cluster bud induction. (2) At 6-BA 0.5–2.0 mg · L ^{1} with IBA or IAA 0.05–0.2 mg · L ^{1}, regenerated buds were primarily single shoots; at 6-BA 4.0 mg · L ^{1}, cluster buds formed but with severe vitrification. (3) Vitrification was problematic in subculture, worsening with higher 6-BA concentrations and increased subculture generations when IBA or IAA exceeded 0.1 mg · L ^{1} [FIGURE:1-B]. (4) Substituting KT or ZT for 6-BA induced excessive callus without bud formation. (5) Using NAA instead of IBA or IAA produced excessive callus, causing gradual shoot chlorosis over time.

Consequently, the viable hormone combination for subculture was 6-BA 0.5 mg · L ^{1} + IBA 0.05 mg · L ^{1}, producing primarily single buds with a moderate proliferation coefficient of 3.5 per 35 days but robust shoots averaging 6.0 cm after 35 days [TABLE:3, FIGURE:1-C and D], suitable for pre-rooting shoot strengthening.

2.3.2 Subsequent Subculture Proliferation Research Preliminary studies identified MS + 6-BA 0.5 mg · L ^{1} + IBA 0.05 mg · L ^{1} as relatively suitable but with a low proliferation coefficient (3.5 per 35 days). Subsequent research adjusted 6-BA:IBA ratios and added TIBA to enhance cluster bud induction and proliferation while controlling vitrification . Comprehensive analysis revealed: (1) After multiple subculture generations, relatively high 6-BA concentrations (0.8 mg · L ^{1}) produced weak shoots with >70% vitrification and low proliferation coefficients. Conversely, low 6-BA (0.2 mg · L ^{1}) prevented vitrification but yielded stunted shoots with low proliferation. At 6-BA 0.5 mg · L ^{1}, shoots were tall and robust, vitrification-free, with relatively high proliferation coefficients, indicating this as the optimal cytokinin concentration for sustained growth. (2)

Among 6-BA:IBA ratios of 20:1, 5:1, and 10:1, the latter produced superior cluster bud rates, shoot heights, and proliferation coefficients. (3) At higher 6-BA (1.0 mg·L ^{1}) and IBA (0.1 mg·L ^{1}) concentrations, TIBA addition at appropriate ratios (1.0:0.1:0.04) reduced vitrification to 20.5%, while inappropriate ratios caused severe vitrification (1.0:0.1:0.06: 80.0%; 1.0:0.1:0.02: 100.0%). Vitrification was thus related not only to individual hormone concentrations but also to their ratios, with optimal combinations significantly suppressing vitrification. (4) At optimal 6-BA (0.5 mg·L ^{1}) and IBA (0.05 mg·L ^{1}) concentrations, TIBA 0.01–0.05 mg·L ^{1} produced cluster bud rates of 65.0–82.3%, but shoots were relatively weak with 20.8–32.0% vitrification and low proliferation coefficients (4.0–5.0 per 35 days). Notably, TIBA at 0.02 mg·L ^{1} achieved 80.5% cluster bud induction with robust shoots averaging 4.5 cm after 35 days, zero vitrification, and a proliferation coefficient of 7.0 per 35 days [TABLE:4, FIGURE:1-E and F], maintaining excellent stability over 10+ subculture generations. The optimal concentration ratio of 6-BA:IBA:TIBA was thus 0.5:0.05:0.02 mg·L ^{1}.

Integrating both subculture phases, the optimal proliferation medium is MS + 6-BA 0.5 mg·L ^{1} + IBA 0.05 mg·L ^{1} + TIBA 0.02 mg·L ^{1} + sucrose 30.0 g·L ^{1} (pH 5.8), yielding 80.5% cluster bud induction, robust shoots averaging 4.5 cm, zero long-term vitrification, and a proliferation coefficient of 7.0 per 35 days. The optimal shoot strengthening medium is MS + 6-BA 0.5 mg·L ^{1} + IBA 0.05 mg·L ^{1} + sucrose 30.0 g·L ^{1} (pH 5.8), producing tall, robust, vitrification-free shoots averaging 6.0 cm after 35 days, suitable for pre-rooting culture.

2.4 Rooting Culture Using 1/2MS and 1/2WPM basal media supplemented with various IBA, NAA, or IAA concentrations, we screened for optimal rooting conditions . Results showed that 1/2MS with IBA failed to induce rooting. In contrast, 1/2WPM with different auxins showed varying effects: NAA at 0.5–4.0 mg·L ^{1} produced 0% rooting with increasing callus formation and browning; IAA at 0.5–4.0 mg·L ^{1} induced rooting with a maximum rate of 20.0% at 2.0 mg·L ^{1}; IBA at 0.5–4.0 mg·L ^{1} also induced rooting, peaking at 55.2% with 1.5 mg·L ^{1}. IBA-IAA combinations did not improve rooting rates. All treatments exhibited leaf tip necrosis (28–50%), which increased with auxin concentration but did not cause plant mortality. The relatively suitable rooting medium was 1/2WPM + IBA 1.5 mg·L ^{1}, though subsequent experiments revealed high inter-batch variability with maximum rooting of only 55.2%.

Therefore, we further tested special rooting promoters DA-6 (diethyl aminoethyl hexanoate) and 5-NGS (5-nitroguaiacol sodium salt) added to 1/2WPM + IBA 1.5 mg·L ^{1} . DA-6 at 1.0–8.0 mg·L ^{1} with IBA 1.5 mg·L ^{1} yielded maximum rooting of only 6.9%, far below IBA alone. In contrast, 5-NGS at 3.0–5.0 mg·L ^{1} with IBA 1.5 mg·L ^{1} showed synergistic effects, with the optimal combination of IBA 1.5 mg·L ^{1} + 5-NGS 4.5 mg·L ^{1} achieving 83.3% rooting, significantly higher than IBA alone (54.6%). The optimal rooting medium is thus 1/2WPM + IBA 1.5 mg·L ^{1} + 5-NGS 4.5 mg·L ^{1}, producing 83.3% rooting after 40 days with robust, healthy plants and reduced leaf necrosis [FIGURE:1-I and J].

However, subsequent experiments confirmed high inter-batch variability, with the highest rooting rates occurring in late November to late December.

2.5 Rooting Seedling Hardening and Transplanting *Cyclocarya paliurus* prefers warm, humid environments with deep, fertile, well-drained acidic soils. Based on these requirements, we tested garden soil, peat soil, and coconut fiber as transplanting substrates during March–June and September–November . After 40 days, survival rates in peat soil and coconut fiber significantly exceeded those in garden soil. Transplanting seasons markedly affected survival, with optimal periods being March–May and October–November (54.3–65.6% survival). During these periods, seedlings developed new roots and leaves, reaching 8–10 cm height after 40 days [FIGURE:1-K].

Discussion and Conclusion

Explant collection timing and type critically influence primary culture of *C. paliurus*. Wang et al. (2016) reported highest axillary bud germination from April-collected stem segments, dropping to zero after August. Fu et al. (2017) found May sampling optimal, with highest germination in the first and second segments below the apex. Our study demonstrates that sampling from April to mid-June, using slightly lignified stem segments disinfected with 0.1% HgCl for 5–7 minutes, achieves favorable disinfection results (54.1% success, 88.7% survival). In contrast, August collections consistently browned and died regardless of treatment, yielding zero survival and primary bud induction. Notably, *C. paliurus* explants are prone to browning during disinfection, with mortality positively correlated with HgCl exposure time, necessitating careful timing control.

Primary culture media composition varies among researchers, yielding different results. Fu et al. (2017) achieved 57.1% bud germination using modified MS + TDZ 0.5 mg · L ^{1} + NAA 0.05 mg · L ^{1}. Xie et al. (2011) obtained 83.3% germination with WPM + 6-BA 3.0 mg · L ^{1} + 2ip 1.0 mg · L ^{1} + NAA 0.1 mg · L ^{1}. Lu et al. (2013) reported axillary bud germination rates up to 86% with appropriate media. Our study found that MS + 6-BA 2.0 mg · L ^{1} + IBA 0.2 mg · L ^{1} yields 80.5% primary bud induction, and that *C. paliurus* primary culture tolerates a wide range of basal media and hormone concentrations.

For subculture proliferation, Lu et al. (2013) found WPM + TDZ 0.3 mg · L ^{1} + 6-BA 0.3 mg · L ^{1} optimal, achieving a proliferation coefficient of 6.8 per 20 days. Hu et al. (2009) obtained numerous proliferated buds using cytokinin ZT. Shang et al. (2007) achieved a maximum proliferation coefficient of 3.45 through embryo culture. Zhang and Fang (2012) and Lu et al. (2013) reported high coefficients (6.71 and 6.78) but with small buds encased in excessive callus, yielding poor subsequent subculture performance. Xie et al. (2011) achieved a proliferation coefficient of 7.3 per 15 days in WPM + TDZ 0.1 mg · L ^{1} + NAA 0.1 mg ·

L ^{1}, finding that GA 2.0 mg/L effectively promoted cluster bud elongation and robust growth. We observed that WPM-based subculture caused leaf chlorosis, while GA addition induced stem node compression, dwarfed shoots, excessive callus, and narrow, curved leaves rather than promoting internode elongation. These discrepancies warrant further investigation. Our experiments revealed that ZT, KT, and NAA induced excessive callus inhibiting shoot regeneration, while vitrification increased with 6-BA concentration and subculture generations. However, insufficient 6-BA could not maintain normal growth. TIBA addition significantly improved cluster bud induction and proliferation coefficients, likely due to its weak anti-auxin activity suppressing apical dominance. Through long-term screening, we identified MS + 6-BA 0.5 mg $\cdot$ L ^{1} + IBA 0.05 mg $\cdot$ L ^{1} + TIBA 0.02 mg $\cdot$ L ^{1} as a stable long-term subculture medium, consistently producing >80% cluster bud induction, zero vitrification, robust shoots, and a proliferation coefficient of 7.0 per 35 days across 10+ generations, suitable for industrial production. Previous literature did not address vitrification or shoot strengthening, possibly due to limited subculture generations. Our findings thus provide valuable reference for long-term subculture and commercial micropropagation of *C. paliurus*.

Rooting studies report IBA as the suitable auxin for *C. paliurus* adventitious root induction. Qiao et al. (2009) found 5.0 mg $\cdot$ L ^{1} IBA yielded maximum rooting (40%). Wang et al. (2009) achieved 37.5% rooting with IBA 0.1 mg $\cdot$ L ^{1} + paclobutrazol 0.5 mg $\cdot$ L ^{1}, though with leaf abscission. Lu et al. (2013) reported 25% rooting with WPM + GGR-6 10.0 mg $\cdot$ L ^{1} + IBA 1.0 mg $\cdot$ L ^{1}. Xie et al. (2009) obtained 16.67% rooting with WPM + IBA 0.2 mg $\cdot$ L ^{1} + sucrose 20 g $\cdot$ L ^{1}, increasing to 23.33% after 15 days dark induction. Zhang et al. (2018) achieved 27.0% rooting with 1/2 MS + IBA 1.5 mg $\cdot$ L ^{1}. These results indicate difficult rooting and low induction rates. We found 1/2MS poorly induced rooting, while 1/2WPM + IBA 1.5 mg $\cdot$ L ^{1} was suitable, and adding 5-NGS 4.5 mg $\cdot$ L ^{1} substantially increased rooting rates. However, high inter-batch variability persists, possibly related to biological clocks and culture seasons, requiring verification.

No literature reports exist on *C. paliurus* tissue culture seedling transplanting. We found these seedlings prone to browning and low survival, with maximum survival only 65.6%, far below industrial requirements. Notably, different *C. paliurus* germplasm sources may respond similarly in primary culture but show distinct genotype-specific requirements during subculture and rooting, likely explaining discrepancies among studies and between our results and published literature.

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