Direct organogenesis and plant regeneration of *Litsea coreana* Levl. var. *sinensis*

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Abstract

Litsea coreana Levl. var. *sinensis* is a versatile evergreen tree species that is difficult to propagate on a large scale using traditional methods. However, research on direct regeneration from stem segments of *L. coreana* var. *sinensis* is limited. This study investigates the disinfection methods for explants, factors influencing axillary bud induction and proliferation, and adventitious root growth. The results indicate that the optimal disinfection method for young stem segments of *L. coreana* var. *sinensis* is 30 s in 75% alcohol followed by 10 min in 0.1% $HgCl_2$. A pre-treatment with 5% carbendazim for 30 min before applying the same disinfection protocol effectively reduces contamination rates in semi-woody stem segments. Adding 1.0 $g \cdot L^{-1}$ activated charcoal (AC) to the axillary bud induction medium significantly decreases browning rates of explants. The suitable basic medium for axillary bud induction is 1/2 MS supplemented with 6-benzylaminopurine (6-BA), while the best hormone combination for inducing buds is 2.0 $mg \cdot L^{-1}$ 6-BA plus 0.3 $mg \cdot L^{-1}$ indolebutyric acid (IBA), achieving an induction rate of 40%. For adventitious shoot proliferation, the optimal medium combination includes 1/2 MS with 1.0 $mg \cdot L^{-1}$ 6-BA, 0.2 $mg \cdot L^{-1}$ kinetin (KT), and 0.5 $mg \cdot L^{-1}$ naphthaleneacetic acid (NAA), resulting in a proliferation rate of 73.33%. Both NAA and IBA can induce rooting in tissue-cultured seedlings; however, adding 0.5 $mg \cdot L^{-1}$ IBA to 1/2 MS yields better rooting results with an induction rate of 43.33%. This study establishes a preliminary direct organogenesis regeneration system for *L. coreana* var. *sinensis*, laying a foundation for further research and development of its clonal propagation techniques.

Key message

The organogenesis regeneration of *L. coreana* var. *sinensis* was established in vitro regeneration of tender shoots and roots. The developed system could be very valuable for its clonal propagation techniques.

Introduction

Litsea coreana var. *sinensis*, also known as eagle tea, is an evergreen tree belonging to the Lauraceae family and is native to Southwest China. It has a drinking history of several hundred years (Yuan et al. 2023). Additionally, it is a medicinal species with high flavonoid content and various reported pharmacological properties (Xie et al. 2025), including antioxidant effects, liver protection, lipid and glucose reduction, anti-inflammatory actions, and immune regulation (Liu et al. 2020).

L. coreana var. *sinensis* is a dioecious species with underdeveloped seeds and a long fruit growth cycle, making its brightly colored fruits attractive to birds. Overharvesting of female trees has hindered their reproductive processes, leading to a sharp decline in seed quantity and difficulties in large-scale propagation through seeds (Pan et al. 2020). Additionally, the *L. coreana* var. *sinensis* is difficult to root; cuttings have low and unstable rooting rates, significantly limiting rapid propagation efficiency (Qu et al. 2022). Therefore, developing an economical and effective propagation method is crucial for resource conservation and sustainable production given these limitations.

Compared to conventional breeding, the organogenesis pathway based on tissue culture technology offers significant advantages for micropropagation, especially for woody plants with low seed germination rates and propagation difficulties (Ahmad et al. 2022). For instance, the related species *Cinnamomum camphora* (Huang et al. 1998) achieved direct organ regeneration using stem segments as explants. Other woody plants with low seed production include *Rhus coriaria* L. (Amiri and Mohammadi 2021), *Quercus ilex* L. (Martínez et al. 2017), and difficult-to-root species such as *Pinus massoniana* (Wan and Fan 2024) and *Morus alba* var. *shidareguwa* (Aroonpong and Chang 2015). Currently, there are few reports on direct organogenesis in *L. coreana* var. *sinensis*, and utilizing this method could effectively address their low propagation efficiency.

In sterile conditions, using tissue culture techniques for in vitro cultivation of plant organs allows for rapid and cost-effective propagation in a limited space. However, the integrity and stability of the culture system are influenced by various factors, including explant type, plant growth regulators, and the type of basic medium (Long et al. 2022); Bernula et al. 2019; Sundararajan et al. 2017). Different stages of cultivation require varying ratios of plant growth regulators. For enhancing bud proliferation rates, an appropriate ratio of cytokinins to auxins is essential, while root induction primarily relies on suitable auxin addition (Uddin et al. 2024).

Currently, there is no research on successfully achieving in vitro regeneration of tender shoots and roots from *L. coreana* var. *sinensis* stem segments through organogenesis. This study uses *L. coreana* var. *sinensis* as material for in vitro propagation, aiming to establish a feasible and effective production method that provides theoretical support for the direct organogenesis of *L. coreana* var. *sinensis.*

Materials and methods

Material source

From March to July 2024, healthy, disease-free young shoots of 5 – year – old *L. coreana* var. *sinensis* plants were collected from the nursery experimental site of Guizhou University's College of Forestry.

Experimental methods

Disinfection treatment

The collected young shoots were washed in water with detergent while scrubbing their surfaces with a brush, then rinsed under running water for 2 hours before being cut into 1.5–2 cm long segments as explants. The disinfection protocol included: (1) soaking in 75% alcohol for 30 s followed by treatment with a 5% NaClO solution for either 15, 20, or 25 minutes; (2) soaking the explants in 75% alcohol for 30 s followed by treatment with a 0.1% $HgCl_2$ solution for either 6, 8, or10 min. For semi – woody stem segments: (1) soak in a 5% carbendazim solution for 30 min, then treat with 75% alcohol for 30 s and subsequently use a 0.1% $HgCl_2$ solution for 7, 10 or 13 min; (2) without pre-treatment using the carbendazim solution, directly soak in 75% alcohol for 30 s and then use a 0.1% $HgCl_2$ solution for 7, 10

or 13 min. All treatment groups contained 20 explants each and were repeated three times. Observations on explant conditions were recorded after 20 days.

Treatment of Explants to Prevent Browning

To investigate the effects of AC and PVP on explant browning, we designed 7 treatment groups: CK, AC (1.0, 1.5, 2.0 $g{\cdot}L^{-1}$), and PVP (1.0, 1.5, 2.0 $g{\cdot}L^{-1}$). The experiment used a half-strength MS medium as the base culture medium, supplemented with 7.5 $g{\cdot}L^{-1}$ agar, 30 $g{\cdot}L^{-1}$ sucrose, and 2.0 m $g{\cdot}L^{-1}$ 6-BA in all formulations. Each treatment group consisted of 20 explants with three replicates. After 20 days, contamination rates, survival rates, and mortality rates were recorded.

Axillary Bud Induction

To investigate the effect of different basal media on axillary bud induction in Cinnamomum molle, stem segments with buds were used as explants and inoculated onto four media: MS, 1/2 MS, 1/4 MS, and WPM. Each treatment group consisted of 20 explants with three replicates. All formulations included 2.0 $mg{\cdot}L^{-1}$ 6 –BA, 7.5 $g{\cdot}L^{-1}$ agar, 30 $g{\cdot}L^{-1}$ sucrose, and AC at 1.0 $g{\cdot}L^{-1}$. After 30 days, the growth of axillary buds was assessed.

To investigate the effects of different cytokinins on axillary bud induction, a 1/2 MS medium was used as the base, supplemented with 7.5 $g{\cdot}L^{-1}$ agar, 30 $g{\cdot}L^{-1}$ sucrose, and 1.0 $g{\cdot}L^{-1}$ AC. Four treatment groups were established, each receiving 2 m $g{\cdot}L^{-1}$ of either 6-BA, TDZ, KT, or ZT. Each group contained 20 explants with three replicates. After 30 days, the axillary bud induction rates were recorded.

To explore the optimal hormone combination for axillary bud induction in *L. coreana* var. *sinensis*, stem segments with buds were selected as explants. After sterilization, they were inoculated onto media containing different concentrations of 6-BA (1.0, 2.0, 3.0) and IBA (0.1, 0.3, 0.5). The medium was based on 1/2 MS and supplemented with 7.5 $g{\cdot}L^{-1}$ agar, 30 $g{\cdot}L^{-1}$ sucrose, and 1.0 $g{\cdot}L^{-1}$ AC. Each treatment group consisted of 30 explants with three replicates to observe and record axillary bud induction. The germination rate was calculated after 30 days.

Axillary Bud Proliferation

To identify the optimal medium for bud proliferation, we used 1/2 MS as the base medium and added different concentrations of 6-BA (1.0, 2.0, 3.0 $mg{\cdot}L^{-1}$), IBA (0.3, 0.4, 0.5 $mg{\cdot}L^{-1}$), and KT (0.1, 0.2, 0.3 $mg{\cdot}L^{-1}$). All formulations included 7.5 $g{\cdot}L^{-1}$ agar and 30 $g{\cdot}L^{-1}$ sucrose. Each treatment group contained ten explants with three replicates. After 60 days, we recorded the rate of adventitious bud proliferation and growth status.

Induction of Adventitious Roots

In the initial culture phase, 1/2 MS medium without hormones was used for sterile cultivation of explants for 30 days. The explants were then transferred to 1/2 MS medium containing different concentrations

of IBA (0.1, 0.5, 1.0 m $g \cdot L^{-1}$) and NAA (0.1, 0.5, 1.0 m $g \cdot L^{-1}$) for root induction. All media included 7.5 $g \cdot L^{-1}$ agar and 30 $g \cdot L^{-1}$ sucrose, with each treatment group consisting of 10 explants and repeated three times. Rooting was assessed after 60 days.

Data Analysis

According to the growth conditions of plant materials, data on sterilization effects of explants were collected after 20 days of culture; axillary bud induction data were recorded after 30 days; and data on adventitious shoot proliferation and root induction were noted after 60 days. SPSS 24.0 software was used for statistical analysis, employing ANOVA combined with Duncan's test to assess differences among groups. A P value < 0.05 indicates significant difference, while a P value > 0.05 indicates no significant difference. Results are presented as mean ± standard error, with all treatments repeated three times.

Results

Disinfection of explants

The comparison of sterilization effects with different disinfection durations (Table 1) shows that the two disinfectants have varying efficacy on the tender stems of *L. coreana* var. *sinensis*, with $HgCl_2$ demonstrating superior results. As the disinfection time with $HgCl_2$ increases, contamination rates decrease while mortality and survival rates improve. The optimal disinfection effect is achieved when treating with 0.1% $HgCl_2$ for 10 minutes, resulting in a minimum contamination rate of 21.67% and a maximum survival rate of 60.00%. Therefore, the recommended disinfection combination for tender stem segments is 75% alcohol for 30 s + 0.1% $HgCl_2$ for 10 min.

Table 1
Effects of different disinfection combinations on the sterilization of young stem segments of *L. coreana* var. *sinensis*

Treatment method	Pollution rate(%)	Mortality rate(%)	Survival(%)
75% alcohol for 30 s, 5% NaClO solution for 15 min	55.00 ± 12.58a	1.67 ± 1.67b	43.33 ± 12.02a
75% alcohol for 30 s, 5% NaClO solution for 20 min	51.67 ± 1.02a	1.67 ± 1.67b	46.67 ± 11.68a
75% alcohol for 30 s, 5% NaClO solution for 25 min	50.00 ± 15.28a	5.00 ± 2.89b	45.00 ± 18.03a
75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 6 min	43.33 ± 4.41a	5.00 ± 2.89b	51.67 ± 6.67a
75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 8 min	35.00 ± 0.00a	8.33 ± 1.67ab	56.67 ± 1.6a
75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 10 min	21.67 ± 6.01a	18.33 ± 6.67a	60.00 ± 5.00a
Note: Same letters indicate no significant difference between treatments ($P > 0.05$), while different letters indicate a significant difference ($P < 0.05$). The same as following.			

From Table 2, it can be observed that the overall contamination rate for semi-lignified stem segments is relatively high. Compared to using only $HgCl_2$ solution, pre-treating semi-lignified stems with a 5% carbendazim solution for 30 minutes before applying $HgCl_2$ yields better results. Based on an analysis of contamination and survival rates, the suitable disinfection treatment combination for semi-lignified stem segments is soaking in a 5% carbendazim solution for 30 min + disinfecting with 75% alcohol for 30 s + treating with $HgCl_2$ for 10 min.

Table 2
Effects of different disinfection combinations on the sterilization of semi-wooded stem segments of *L. coreana* var. *sinensis*

Treatment method	Pollution rate(%)	Mortality rate(%)	Survival rate(%)
5% carbendazim solution for 30 min, 75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 7 min	83.33 ± 4.41a	5.00 ± 2.89bc	11.67 ± 1.67ab
5% carbendazim solution for 30 min, 75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 10 min	76.67 ± 7.27a	5.00 ± 2.89bc	18.33 ± 6.01a
5% carbendazim solution for 30 min, 75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 13 min	76.67 ± 3.33a	16.67 ± 1.67a	6.67 ± 1.67b
75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 7 min	88.33 ± 1.67a	0.00 ± 0.00c	11.67.00 ± 1.67ab
75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 7 min.	81.67 ± 1.67a	1.67 ± 1.67c	16.67 ± 1.67ab
75% alcohol for 30 s, 0.1% $HgCl_2$ solution for 13 min.	75.00 ± 2.89a	11.67 ± 1.67ab	13.33 ± 4.41ab

Effects of different concentrations of browning inhibitors on explant browning

During the culture of explants in axillary bud induction medium, significant browning was observed, severely affecting subsequent experiments. Therefore, it is essential to explore effective inhibitors to ensure normal growth of explants. As shown in Table 3, increasing the amounts of AC and PVP led to a gradual decrease in browning rates. The addition of AC significantly inhibited explant browning, while PVP showed minimal effect. Compared to the control CK, the lowest browning rate occurred with 2.0 $g{\cdot}L^{-1}$ AC added. However, studies indicate that AC exhibits non-selective adsorption properties; it adsorbs both the brown substances secreted by explants and nutrients from the medium, potentially impacting axillary bud induction effectiveness. In this study, there was no significant difference in browning rates between adding 1.0 $g{\cdot}L^{-1}$ AC and higher concentrations (1.5 and 2.0 $g{\cdot}L^{-1}$). Therefore, considering its effectiveness in controlling browning without harming explants, we chose to add 1.0 $g{\cdot}L^{-1}$ AC for further research on axillary bud induction (Fig. 1A-c).

Table 3
The effect of different concentrations of anti-browning agents on the browning of stem segment explants of *L. coreana* var. *sinensis*

Treatment	Inoculation count	Browning Count	Browning Rate(%)
CK	60	37	61.67 ± 10.14 a
AC (1.0 $g \cdot L^{-1}$)	60	9	15.00 ± 5.78b
AC (1.5 $g \cdot L^{-1}$)	60	8	13.33 ± 6.01b
AC (2.0 $g \cdot L^{-1}$)	60	3	5.00 ± 2.89b
PVP (1.0 $g \cdot L^{-1}$)	60	25	41.67 ± 11.67ab
PVP (1.5 $g \cdot L^{-1}$)	60	25	41.67 ± 8.82 ab
PVP (2.0 $g \cdot L^{-1}$)	60	21	35.00 ± 7.64 ab

Induction culture of axillary buds

Effects of different basic media on axillary bud induction

This study revealed significant differences among four basic media in promoting axillary bud germination (Table 4). The induction effect of axillary buds from the stem segments was poorest on WPM medium, requiring the longest time of 30 days for induction. Compared to 1/2 MS and 1/4 MS media, MS medium also showed relatively poor results with an induction time of 20 days. Both 1/2 MS and 1/4 MS media facilitated faster axillary bud induction with healthy seedlings that had vibrant green leaves. Overall, the shortest germination time was achieved using the 1/2 MS formulation at just 14 days, resulting in well-growing plants. Considering both induction efficiency and growth status, the most suitable basic medium for axillary bud induction is identified as 1/2 MS.

Table 4
Effects of four basic media on axillary bud induction in *L. coreana* var. *sinensis*

Basic medium	6-BA (m g·L^{-1})	AC (g·L^{-1})	Germination time	Growth description
MS	2.0	1.0	20 d	The leaves are green, and the axillary buds grow slowly (Fig. 1B-a).
1/2MS	2.0	1.0	14 d	The leaves are green, and the axillary buds grow quickly (Fig. 1B-b).
1/4MS	2.0	1.0	18 d	The leaves are green, and the axillary buds grow quickly. (Fig. 1B-c)
WPM	2.0	1.0	30 d	Axillary bud growth is slow (Fig. 1B-d).

Effects of different cytokinins on axillary bud induction

During axillary bud induction, cytokinins promote cell division and stimulate bud sprouting, but their effectiveness varies by species (Table 5). To identify suitable hormones for inducing axillary buds in *L. coreana* var. *sinensis*, four cytokinins were compared: 6-BA, TDZ, KT, and ZT at a concentration of 2.0 mg·L^{-1} in 1/2 MS medium. Significant differences were observed among the treatments, with the induction rates ranked as follows: 6-BA > KT > ZT > TDZ. Notably, 6-BA showed the highest adaptability with an induction rate of 21.67%, while TDZ resulted in the lowest rate at 13.33%.

Table 5
Effects of four cytokinins on axillary bud induction in *L. coreana* var. *sinensis*

Treatment	Type	Concentration (mg·L^{-1})	Germination time
MS	2.0	1.0	21.67 ± 1.67a
1/2 MS	2.0	1.0	13.33 ± 1.31c
1/4 MS	2.0	1.0	20.00 ± 3.33ab
WPM	2.0	1.0	15.00 ± 0.00bc

Effects of hormone concentration ratios on axillary bud induction

After sterilizing the stem segments of *L. coreana* var. *sinensis*, they were inoculated onto 1/2 MS medium with different concentrations of 6-BA and IBA for axillary bud induction. The induction effects

showed significant differences (Table 6). As the concentration of 6-BA increased, the axillary bud induction rate initially rose and then fell, peaking at 2.0 mg·L^{-1}. At this concentration, the induction rate also followed a similar trend with increasing IBA levels, reaching its maximum at an IBA concentration of 0.3 mg·L^{-1}. The optimal hormone combination for axillary bud induction was found to be: 2 mg·L^{-1} 6-BA + 0.3 mg·L^{-1} IBA, achieving an induction rate of up to 40.00%.

Table 6
Effects of 6-BA and IBA concentration ratio on axillary bud induction in *L. coreana* var. *sinensis*

6-BA (m g·L^{-1})	IBA (m g·L^{-1})	Induction rate(%)
1.0	0.1	17.78 ± 2.94b
1.0	0.3	18.89 ± 4.00b
1.0	0.5	24.44 ± 2.94b
2.0	0.1	31.11 ± 2.94ab
2.0	0.3	40.00 ± 3.33a
2.0	0.5	32.22 ± 7.78ab
3.0	0.1	20.00 ± 5.09b
3.0	0.3	24.44 ± 4.84b
3.0	0.5	18.89 ± 5.88b

Variance analysis results indicated that there were highly significant differences in axillary bud induction rates due to varying concentrations of 6-BA (Table 7). Both excessively high and low concentrations inhibited the induction effect significantly. However, no significant difference was observed with varying IBA levels, indicating that 6-BA plays a primary role in axillary bud induction.

Table 7
Variance analysis of the effect of 6-BA and IBA concentration ratio on axillary bud induction in *L. coreana* var. *sinensis*

Source of difference	Sum of Squares	Df	Mean Square	F ratio	Sig.
Intercept	1.788	1	1.788	267.265	0.000**
6-BA	0.124	2	0.062	9.277	0.001**
IBA	0.012	2	0.006	0.871	0.432
Error	0.147	22	0.007		
Total	2.071	27			
Note: * indicates $P < 0.05$ for significant differences; ** indicates $P < 0.01$ for highly significant. The same as following.					

Axillary bud proliferation

The induced adventitious buds were cultured for proliferation, revealing that different concentrations of 6-BA, IBA, and KT significantly affect their proliferation rates. Results showed variations in bud proliferation rates across treatments (Table 8). As the concentration of 6-BA increased, the proliferation rate gradually decreased. The best overall bud growth was observed at a 1.0 $mg{\cdot}L^{-1}$ concentration of 6-BA. The optimal combination identified was: 1.0 $mg{\cdot}L^{-1}$ 6-BA + 0.2 $mg{\cdot}L^{-1}$ KT + 0.5 $mg{\cdot}L^{-1}$ NAA, achieving a proliferation rate of 73.33% with abundant bud formation (Fig. 1-D).

Table 8
Effects of 6-BA and IBA concentration ratio on axillary bud induction in *L. coreana* var. *sinensis*

6-BA ($mg{\cdot}L^{-1}$)	NAA ($mg{\cdot}L^{-1}$)	KT ($mg{\cdot}L^{-1}$)	Growth description
1.0	0.3	0	50.00 ± 0.00 bc
1.0	0.4	0.1	67.67 ± 3.33 ab
1.0	0.5	0.2	73.33 ± 6.67 a
2.0	0.3	0.1	43.33 ± 5.77 cd
2.0	0.4	0.2	30.00 ± 3.33 ef
2.0	0.5	0	33.33 ± 8.82 cd
3.0	0.3	0.2	36.37 ± 3.33 cd
3.0	0.4	0	13.33 ± 3.33 e
3.0	0.5	0.1	50.00 ± 0.00 bc

Variance analysis indicated that both 6-BA and KT have significant effects on the proliferation of *L. coreana* var. *sinensis* adventitious buds, while NAA did not show a significant impact, highlighting the crucial roles of 6-BA and KT in this process (Table 9).

Table 9
Variance analysis of the effects of 6-BA, IBA, and KT concentration ratios on adventitious bud proliferation from *L. coreana* var. *sinensis*

Source of difference	Sum of Squares	df	Mean Square	F ratio	Sig.
Intercept	5.229	1	5.229	454.996	0.000**
6-BA	0.917	2	0.459	39.912	0.000**
NAA	0.037	2	0.018	1.602	0.226
KT	0.158	2	0.079	6.860	0.005**
Error	0.230	20	0.011		
Total	6.751	27			

Induction of indeterminate roots

The tissue culture seedlings were transferred to 1/2 MS medium containing different concentrations of IBA and NAA for cultivation, resulting in varying induction and growth of adventitious roots (Table 10). Roots were induced at 0.1 mg·L^{-1} of NAA and IBA. However, they were successfully induced at concentrations of 0.5 mg·L^{-1} and 1.0 mg·L^{-1}. The addition of 0.5 mg·L^{-1} NAA and IBA resulted in better root growth. Among all treatments, the highest rooting rate was observed with 0.5 mg·L^{-1} IBA, reaching 46.67%.

Table 10
Effects of IBA and NAA on adventitious root formation

IBA (m g·L^{-1})	NAA (mg·L^{-1})	Rooting rate(%)	Number of rooting
0.1	0.0	0.00 ± 0.00d	0.00 ± 0.00c
0.5	0.0	43.33 ± 3.33a	1.87 ± 0.20ab
1.0	0.0	13.33 ± 3.33c	1.33 ± 0.33ab
0.0	0.1	0.00 ± 0.00d	0.00 ± 0.00c
0.0	0.5	33.33 ± 3.33b	2.17 ± 0.25a
0.0	1.0	6.67 ± 3.33cd	1.00 ± 0.58bc

Discussion

Disinfection of explants and anti-browning treatment

One of the challenges in plant tissue culture is microbial contamination during the early stages of propagation. Therefore, a critical step in research is surface sterilization of plant materials to determine the most suitable disinfection method (Nas and Eşitken 2023). This is especially important for woody

plants, as selecting an appropriate disinfection method is key to successful in vitro culture(Ahmadpoor et al. 2022). Common disinfectants include NaClO, alcohol, $HgCl_2$, and carbendazim (Wan and Fan 2024). Among these, $HgCl_2$ has excellent sterilization efficacy (Aroonpong and Chang 2015) and is widely used in tissue culture experiments. However, it is more toxic than other disinfectants. Additionally, using a combination of multiple disinfectants often yields better sterilization results compared to single agents (Rafiq et al. 2021). The sensitivity of explants to disinfectants varies based on factors such as tenderness and developmental stage. Optimizing disinfection protocols involves adjusting the ratios and exposure times of selected agents according to explant characteristics. For tender stem segments of Cinnamomum molle, this study employed 75% alcohol, 0.1% $HgCl_2$, and 5% NaClO as disinfectants. A combined treatment using a pre-soak with 75% alcohol for 30 s followed by immersion in a solution of 0.1% $HgCl_2$ for 10 min significantly improved surface sterilization efficiency—likely due to alcohol disrupting microbial membranes which enhances mercury ion penetration for bactericidal action. Research indicates that younger plant materials have lower contamination rates and greater cell proliferation potential compared to mature tissues (Valledor et al. 2010). Following approach with carbendazim on *Pinus massoniana* buds (Wan and Fan 2024). This study optimized disinfection methods for semi-woody stem segments by first soaking them in a solution containing 5% carbendazim for thirty minutes before applying a sequential treatment with 75% alcohol for 30 s followed by 10 minutes with 0.1% $HgCl_2$. This resulted in superior disinfection compared to using only $HgCl_2$.

The browning phenomenon in woody plants is influenced by the content of certain compounds within their tissues (Aroonpong and Chang 2015). Among these, phenolic acids and flavonoids are closely related to plant browning (Samadi et al. 2021). The bark of *L. coreana* var. *sinensis* contains abundant phenolic compounds (Xie et al. 2025), which can be released from the cut ends of explants, oxidizing the tissue and causing browning in the culture medium (Vrundha and Thomas 2023). In this study, different concentrations of AC significantly inhibited explant browning. However, AC also non-selectively adsorbs nutrients from the medium, which may hinder plant growth (Thomas 2008). Therefore, based on its effectiveness in controlling browning without harming explants, we recommend adding 1.0 $g{\cdot}L^{-1}$ AC to axillary bud induction media for optimal results.

Axillary bud induction

The basic culture medium is essential for maintaining the growth, differentiation, and development of plants in vitro (Jayusman1 et al. 2022). Studies by Liu et al (2023) on *Diospyros oleifera* and Gu et al (2022) on *Cnidoscolus aconitifolius* showed that a low concentration of trace elements in 1/2 MS medium yielded superior bud induction results, consistent with our findings. In this experiment, four types of basic media were compared. Results indicated that all could stimulate axillary bud growth but differed significantly in effectiveness: 1/2 MS > 1/4 MS > MS > WPM. This contrasts with most studies on woody plants which suggest that MS and WPM are optimal for induction. For instance, Kharel et al (2022) found WPM to be the best medium for axillary bud induction in *Vaccinium corymbosum* L. These

findings highlight significant differences in nutrient requirements during tissue culture processes due to unique genetic traits and biological characteristics of each plant species (Hazrati et al. 2022).

Due to the insufficient supply of endogenous hormones for growth in explants, it is essential to supplement plant growth regulators during tissue culture to ensure normal development (Oberschelp et al. 2015). Cytokinins promote cell proliferation and differentiation (Hou et al. 2023) by influencing cell division patterns and meristem activity, triggering adventitious bud formation (González-Rodríguez et al. 2010). Ram et al (2022) found that optimal axillary bud induction occurs in media supplemented with TDZ. (Adugna et al. 2020) reported better bud induction when explants were cultured on media with varying concentrations of KT. In this study, 6-BA was identified as the most effective cytokinin for axillary bud induction, significantly outperforming the KT, ZT and TDZ in terms of induction rate and growth vigor.

Auxins and cytokinins exhibit significant synergistic effects in regulating plant growth and development (Hussain et al. 2019). This is similar to the findings of Wan and Fan (2024) on *Pinus massoniana*, where the addition of 2 $mg{\cdot}L^{-1}$ 6-BA and 0.1 $mg{\cdot}L^{-1}$ IBA effectively promoted axillary bud germination. A similar phenomenon was observed in the induction experiments of adventitious buds in *Bambusa maculata* (Larekeng et al. 2020), further confirming the synergistic effect of these two hormones. Notably, some studies indicate that cytokinins outperform auxins in stimulating axillary bud germination (Ndlovu et al., 2024). Our results show that adding 2 $mg{\cdot}L^{-1}$ 6-BA and 0.1 $mg{\cdot}L^{-1}$ IBA significantly enhances bud induction, consistent with previous research conclusions.

Indeterminate bud proliferation

Increasing the concentration of cytokinins while reducing auxin levels significantly enhances the proliferation efficiency of indeterminate buds (Sultana et al. 2022). In this study, both 6-BA and KT had a significant impact on the proliferation of indeterminate buds in *L. coreana* var. *sinensis*, with 6-BA showing a greater effect than KT. As previous studies have indicated, 6-BA plays a crucial role in promoting cell division and proliferation in plant tissue cultur e(Xiong et al. 2021). For instance, it has shown notable promoting effects during asexual reproduction through direct organ utilization in woody plants such as *Gardenia jasminoides* (Ai et al. 2024) and *Populus trichocarpa* (Nas and Eşitken 2023). However, excessively high concentrations of 6-BA can inhibit stem growth. This observation aligns with our findings from *L. coreana* var. *sinensis* tissue culture: low concentrations of 6-BA significantly enhance bud regeneration efficiency, whereas high concentrations lead to reduced proliferation rates and poor bud vigor.

Induction of adventitious roots

The formation of adventitious roots is a critical stage in plant growth and development, directly affecting survival rates and subsequent growth (Amirchakhmaghi et al. 2019). Auxins, as essential hormones for promoting adventitious root formation, stimulate cell division and activate root meristematic tissue, playing a decisive role in rooting of tissue-cultured seedlings (Sekhar et al. 2011; Díaz-Sala 2014). Different auxins have varying physiological effects, leading to different induction efficiencies for

adventitious roots. Numerous studies indicate that IBA and NAA are widely used as optimal rooting hormones in tissue culture. The addition of NAA during rooting can stimulate the production of callus and roots (Nakano et al. 1999). Showing superior adaptability in *Moringa oleifera* (Zhang et al. 2017) and *Eucalyptus bosistoana* (Shwe and Leung 2020); IBA demonstrate better adaptability in *Sapium sebiferum* (Hou et al. 2020) and *Begonia homonyma* (Kumari et al. 2017). This study found differences between IBA and NAA in inducing roots from *L. coreana* var. *sinensis*. At low concentrations, neither IBA nor NAA effectively promoted adventitious root formation. However, at higher concentrations, both auxins exhibited significant rooting effects with IBA being notably more effective than NAA. This difference may be influenced by the levels of endogenous auxin, the amount of free auxin available to responsive cells, and the affinity of auxin receptors involved in the rooting response (Park et al. 2017).

Conclusion

The article achieved regeneration using *L. coreana* var. *sinensis* stem segments as explants through direct organogenesis. The appropriate surface disinfection method for semi-woody stem segments of *L. coreana* var. *sinensis* is soaking in 75% alcohol for 30 s, followed by immersion in 0.1% $HgCl_2$ for 10 min. Based on this, soaking in 5% carbendazim for 30 min effectively reduces contamination rates. Adding 1.0 $g{\cdot}L^{-1}$ AC to the induction medium can significantly decrease browning of explants. Results indicate that the optimal combination for axillary bud induction is using 2.0 $mg{\cdot}L^{-1}$ BA and 0.3 $mg{\cdot}L^{-1}$ IBA in 1/2 MS medium. For adventitious root induction, adding IBA at a concentration of 0.5 m $g{\cdot}L^{-1}$ to 1/2 MS medium yields a relatively high induction rate.

Declarations

Author contributions LMY performed the experiments and wrote the manuscript. GQQ and LHE supervised the research and revised the manuscript. YL and XY participated in the modification of the manuscript. FC and MZH performed the plant management and analyzed the data. All authors have read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this article.

Conflicts of interest The authors declare that they have no relevant financial or non-financial interests to disclose.

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Figures

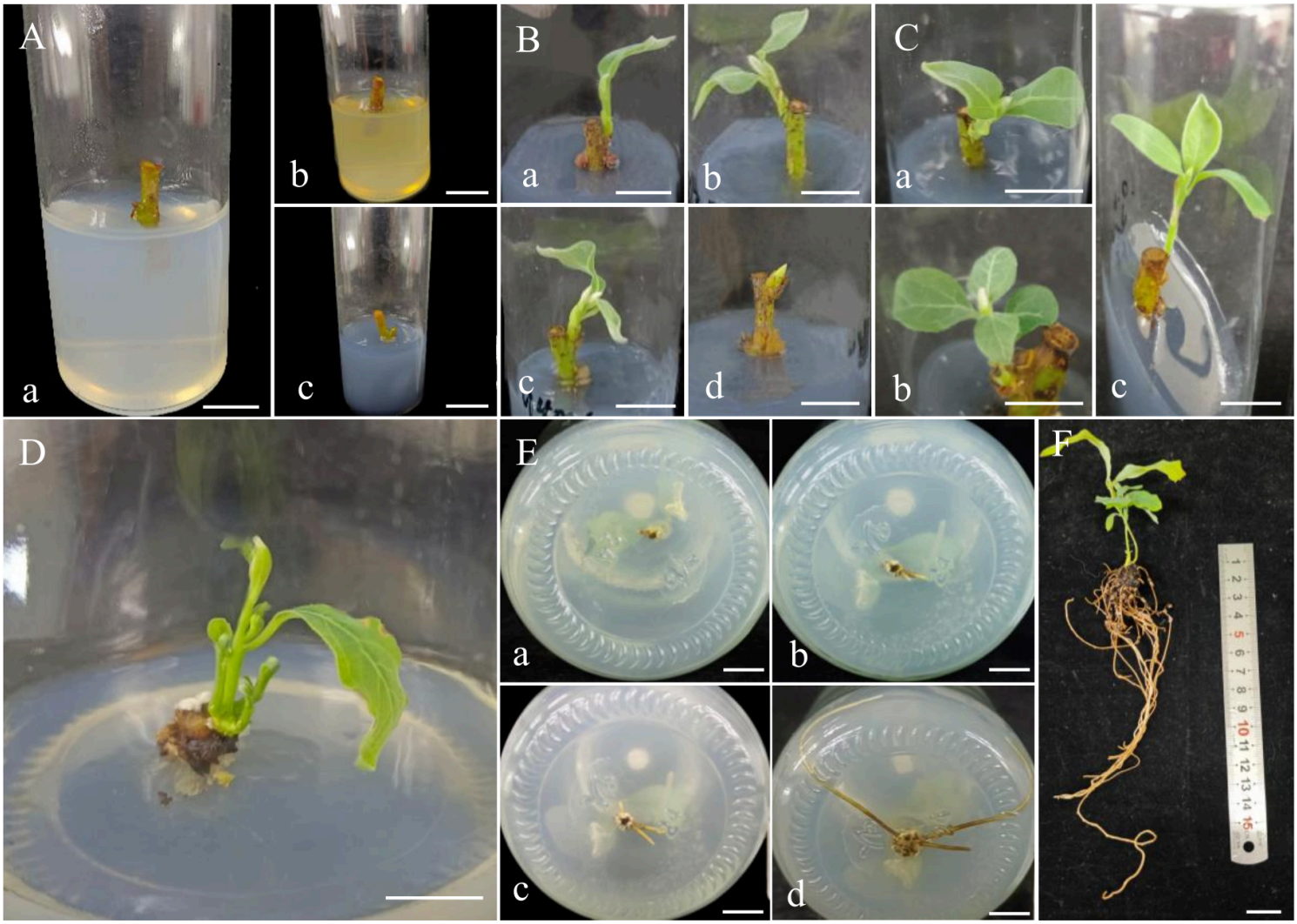

Figure 1

In vitro direct shoot organogenesis and adventitious root induction of *L. coreana* var. *sinensis*.

(A) Induced surface-disinfected stem segments in the culture medium. a. Stem segments at 0 days post-inoculation; b. Browned stem segments after one week of inoculation; c. Stem segments added to the medium at 1.0 $g \cdot L^{-1}$ after one week of inoculation. (B) Induction of axillary buds on four basic media for 4 weeks: a. Axillary buds induced on MS medium; b. Axillary buds induced on 1/2 MS medium; c. Axillary buds induced on 1/4 MS medium; d. Axillary buds induced on WPM medium. (C) Induction of axillary buds on 1/2 MS with 6-BA (2.0 $mg \cdot L^{-1}$) and IBA (0.3 $mg \cdot L^{-1}$): a. Buds induced for 4 weeks; b-c. Buds induced for 6 weeks. (D) Induction of axillary buds on 1/2 MS with 6-BA (2.0 $mg \cdot L^{-1}$), KT (0.2 $mg \cdot L^{-1}$), and NAA (0.5 $mg \cdot L^{-1}$). (E) Adventitious root induction on 1/2 MS with NAA (0.5 $mg \cdot L^{-1}$). a. Roots induced for 4 weeks; b. Roots induced for 5 weeks; c, d: Roots induced for up to 8 weeks. Bar=1 cm