

Establishment of an Efficient Tissue Culture and Regeneration System for *Lycium chinense* var. *macrophyllum* and Its Callus Culture Characteristics

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Abstract

Lycium chinense var. *macrophyllum*, a unique Chinese medicinal and edible plant with high nutritional value and broad application prospects, is limited by inefficient traditional breeding methods. Taking different tissues as explants, this study explored 6-BA and NAA ratio effects on tissue culture and optimized disinfection. The results showed that the optimal disinfection procedure was rinsing with running water for 2 h, soaking in 75% ethanol for 60 s, after rinsing with sterile water, sterilizing with 1% sodium hypochlorite for 20 min. The optimal media for callus induction, bud differentiation and root induction were MS + 6-BA 0.3 mg/L + NAA 1.0 mg/L, MS + 6-BA 0.3 mg/L + NAA 0.5 mg/L, and MS + NAA 0.2 mg/L, respectively. After acclimatization, the survival rate of transplanted regenerated plants reached 95%. This study established an efficient tissue culture and regeneration system, providing technical support for its germplasm preservation and genetic improvement.

1. Introduction

Lycium chinense var. *macrophyllum* are valuable resources with both medicinal and edible purposes, offering medicinal, culinary, and economic value. They are rich in bioactive compounds and widely used in food processing and pharmaceuticals[1–2]. Large-leaved goji, as a high-quality vegetable and medicinal variety of the *Lycium* genus, has broad, thornless leaves, high edibility, and strong tolerance to shade and drought, with wide adaptability, making it suitable for cultivation in both northern and southern regions. It has potential for both cultivation promotion and industrial development. However, traditional propagation methods relying on cuttings and root division have low reproductive efficiency, easy germplasm degradation, and difficulty in stably inheriting superior traits, severely limiting large-scale production and efficient resource utilization[3].

Plant tissue culture technology, with its advantages of being unaffected by seasons, fast propagation, and the ability to maintain excellent varietal traits, has become a core technology for the rapid propagation, germplasm conservation, and genetic improvement of *Lycium* species. Currently, research on tissue culture of *Lycium* species has made certain progress, with rapid propagation systems established for varieties such as large-fruited *Lycium chinense* Mill. and *Lycium ruthenicum*. Notably, for black goji, an optimized leaf co-culture system with high genetic transformation efficiency and low seedling vitrification rate has been developed, providing an important reference for tissue culture research of large-leaf goji[4–5].

Callus induction and culture are core steps in plant tissue culture and also form the foundation for subsequent cell suspension culture, genetic transformation, and secondary metabolite extraction[6]. Existing studies indicate that the type and ratio of plant growth regulators, such as NAA and 6-BA, are key factors affecting the induction, proliferation, and differentiation of callus in *Lycium* species. For instance, research on the induction of friable embryogenic callus in Ningxia goji has further clarified the characteristics of embryogenic callus culture, providing technical reference for the induction of callus in large-leaved goji [7–9].

Although research on tissue culture of *Lycium* species has gradually deepened, specialized studies on large-leaved goji (*Lycium chinense* var. *macrophyllum*) are still lacking. Therefore, conducting research on tissue culture and callus of large-leaved goji, optimizing explant selection, medium formulation, and culture conditions, and identifying the key factors for callus induction and differentiation, is crucial for establishing an efficient and stable tissue culture system. This not only addresses the bottlenecks of traditional propagation and enables rapid propagation and preservation of superior germplasm, but also lays the foundation for genetic improvement, secondary metabolite development, and industrial utilization. It holds significant theoretical and practical importance for promoting the diversified development of *Lycium* plant resources.

2. Results

2.1. Disinfection pretreatment of tender *Lycium chinense* Mil.

The result showed that the duration of ethanol and sodium hypochlorite (NaClO) treatment were significant differences in surface sterilization effects on large-leaved goji seedlings, and also affected the decontamination rate and germination rate (Fig. 1, Table 1). When washed with running water for 30 minutes, treated with ethanol for 8 minutes, and treated with (8%-10%) sodium hypochlorite for 10–15 minutes, the sterilization rate remained at a low level (0%-50%), indicating that the duration and concentration of ethanol and sodium hypochlorite were insufficient to completely remove bacterial contaminants. In comparison, running water washing for 2 hours, ethanol treatment (75%, 1 minute), and sodium hypochlorite treatment (1%, 15–20 minutes) achieved higher sterilization rates (80%-100%). Both 15min and 20min sodium hypochlorite treatments achieved 100% germination rates and were initially free of contamination, but seeds treated with sodium hypochlorite for 15 minutes began to show contamination after 8 weeks of cultivation. Therefore, ethanol treatment for 1 minute and sodium hypochlorite treatment for 20 minutes were confirmed as the optimal scheme, which ensured high disinfection efficiency while retaining seed germination ability. This optimized scheme will be applied in subsequent research.

Table 1
Effects of Different Disinfection Methods on *Lycium chinense* var. *macrophyllum*

Treatments	1st Disinfection	2nd Disinfection	Number of Washes	Disinfection Rate (%)	Germination Rate (%)
1	70%C ₂ H ₅ OH(8min)	8%NaClO(10min)	3	20%	10%
2	70%C ₂ H ₅ OH(8min)	8%NaClO(15min)	3	20%	0%
3	75%C ₂ H ₅ OH(8min)	8%NaClO(10min)	3	30%	5%
4	75%C ₂ H ₅ OH(8min)	8%NaClO(15min)	3	50%	10%
5	75%C ₂ H ₅ OH(8min)	10%NaClO(10min)	3	40%	0%
6	75%C ₂ H ₅ OH(8min)	10%NaClO(15min)	3	40%	0%
7	75%C ₂ H ₅ OH(3min)	8%NaClO(10min)	3	80%	60%
8	75%C ₂ H ₅ OH(0.5min)	1%NaClO(15min)	6	95%	95%
9	75%C ₂ H ₅ OH(1min)	1%NaClO(20min)	6	95%	95%

2.2. Effects of Different Ratios of NAA and 6-BA on the Induction of Callus in *Lycium chinense* var. *Macrophyllum* Leaves

There are significant differences in the induction effect of callus in large-leaved goji leaves with different concentration ratios of naphthaleneacetic acid (NAA) and 6-benzylaminopurine (6-BA). As shown in Table 2 and Fig. 2, adding NAA alone (medium C) or a high concentration of 6-BA alone (medium E) cannot induce callus formation, indicating that callus formation in *Lycium chinense* var. *Macrophyllum* leaves depends on the synergistic action of auxins and cytokinins. Among the combinations with both hormones added, medium D (6-BA 0.3 mg/L, NAA 1.0 mg/L) showed the highest callus induction (+++), with the callus appearing pale yellow, loose in texture, and vigorous in growth, significantly better than medium A (+, with fewer and denser callus) and medium B (+, with slow callus growth). From this, the approximate optimal ratio range is preliminarily determined to be 6-BA 0.2–0.4 mg/L and NAA 0.8–1.2 mg/L(Fig. 3).

Table 2

The effect of different ratios of NAA and 6-BA on the induction of callus in *Lycium chinense* var. *macrophyllum*

Treatments	6-BA (mg/L)	NAA (mg/L)	Presence of callus tissue	Degree of callus induction
A	0.3	0.05	Yes	+
B	0.5	0.5	Yes	+
C	-	0.2	No	-
D	0.3	1	Yes	+++
E	1	0.2	No	-

To more accurately determine the optimal concentration, this study conducted a concentration gradient experiment within the above-mentioned concentration range (Table 3 and Fig. 4). The results showed that with the synergistic increase of 6-BA and NAA concentrations, the induction rate of callus tissue generally showed an upward trend. When the 6-BA concentration was 0.4 mg/L and the NAA concentration was 1.2 mg/L (medium H), the induction rate reached its peak (84%), and the degree of browning was minimal. At this point, the callus was light yellow-green, loosely structured, grew well, and exhibited the best overall effect. In comparison, although medium E (6-BA 0.3 mg/L, NAA 1.2 mg/L) also had a relatively high induction rate (70%), its degree of browning was higher, with localized browning and hardening of the callus, which adversely affected subsequent subculturing.

Table 3
Effect of different concentration gradients of NAA and 6-BA on the induction of callus in *Lycium chinense* var. *macrophyllum*

Treatments	Culture Medium(mg/L)	Number of explants	Number of calluses	Induction rate	Browning condition
A	MS + 6-BA 0.2 + NAA 0.8	20	9	45%	+
B	MS + 6-BA 0.2 + NAA 1.0	20	11	55%	+
C	MS + 6-BA 0.2 + NAA 1.2	20	10	50%	++
D	MS + 6-BA 0.3 + NAA 0.8	20	11	55%	++
E	MS + 6-BA 0.3 + NAA 1.2	20	14	70%	+++
F	MS + 6-BA 0.4 + NAA 0.8	25	14	56%	+++
G	MS + 6-BA 0.4 + NAA 1.0	20	10	50%	++
H	MS + 6-BA 0.4 + NAA 1.2	25	21	84%	+

In conclusion, the preliminary screening in Table 2 and the gradient verification in Table 3 clearly indicate that 6-BA at 0.4 mg/L combined with NAA at 1.2 mg/L is the optimal hormone combination for inducing callus formation in *Lycium chinense* var. *macrophyllum*. This combination not only achieves the highest induction rate but also effectively controls browning, providing a solid foundation for subsequent proliferation and differentiation.

2.3 Effects of Plant Growth Regulators on Bud Induction

This study investigated the regulatory effects of different concentration combinations of cytokinin (6-BA) and auxin (NAA) on bud differentiation in large-leaf goji stem segments. The results showed that there were significant differences in bud induction efficiency (Table 5, Fig. 5). Treatment with 0.3 mg/L 6-BA and 0.05 mg/L NAA (Medium A) showed the best bud induction, with an average of 8 buds per explant and an induction rate of 80%, and the explants exhibited only slight browning. In contrast, treatment with 0.5 mg/L 6-BA and 0.5 mg/L NAA (Medium B) resulted in a significantly lower induction rate of only 10%, an average of 1 bud per explant, and increased explant browning. When treating with 0.8 mg/L 6-BA and 0.05 mg/L NAA (Medium C) completely inhibited bud formation, with a 0% induction rate and severe browning of the explants.

In addition, SSR multiple comparison results also showed that the number of buds and induction rate in Medium A were significantly higher than those in Media B and C ($P < 0.01$), while there was no significant

difference between Media B and C ($P > 0.05$). This indicates that a combination of low concentrations of 6-BA and NAA is more conducive to bud induction in large-leaf goji stem segments, whereas high concentrations of 6-BA or NAA inhibit bud differentiation and exacerbate explant browning.

Table 5

Effect of different ratios of NAA and 6-BA on bud induction of *Lycium chinense* var. *macrophyllum* stem segments Note: Data in the same column with the same letter indicate no statistically significant difference according to the SSR test ($P \geq 0.05$); different letters indicate significant differences (lowercase, $\alpha = 0.05$) or highly significant differences (uppercase, $\alpha = 0.01$); browning conditions: slight browning(+), severe browning(++).

Treatments	Culture Medium(mg/L)	Number of explants	of induced buds	Induction rate(%)	$\alpha = 0.05$	$\alpha = 0.01$	Browning condition
A	MS + 6-BA 0.3 + NAA 0.05	10	0	0%	a	A	+
B	MS + 6-BA 0.5 + NAA 0.5	10	1	10%	b	B	++
C	MS + 6-BA 0.8 + NAA 0.05	10	8	80%	b	B	++

2.4 Effects of Plant Growth Regulators on Root Induction

The figure showed the rooting phenotype of large-leaf goji tissue-cultured seedlings after 4 weeks of culture on MS medium supplemented with 0.2 mg/L α -naphthaleneacetic acid (NAA)(Fig. 6). The left image is a side view, from which it can be observed that the aboveground part of the tube seedlings is growing well, with leaves displaying a fresh green color and a fully expanded shape, without yellowing or vitrification, indicating that this concentration of NAA does not inhibit stem and leaf growth. Several white young roots are produced at the base of the stem, with root lengths of about 0.5–1.0 cm. The right image is a top view, clearly showing green callus (diameter about 2–3 mm) formed at the base of the stem, from which 3–5 root primordia and young roots differentiate, with a radial distribution of the root system. This phenotype is highly consistent with the experimental data of rooting rate (85%) and average number of roots (4.2 roots/plant), visually confirming that 0.2 mg/L NAA can effectively induce root primordium formation and root system development in large-leaf goji tissue-cultured seedlings, without significant phytotoxic effects, making it the optimal concentration for in vitro root induction of this species.

2.5 Acclimatization of tissue cultured *Lycium chinense* seedlings

After rooting, seedlings were transferred to small plastic pots. The acclimatization substrate was selected as imported peat mixed with vermiculite at a volume ratio of 3:1. First, open the sterile bottles to allow the seedlings a 24-hour adaptation period, then transplant the seedlings into small plastic pots filled with the above mixture at 65% humidity, and cultivate the seedlings in an intelligent greenhouse

with a controlled temperature of 25°C. Statistics showed that when using the imported Pindstrup peat and vermiculite mixture as the substrate, the seedling survival rate reached 95%. This result indicates that a substrate with good aeration and balanced water retention is beneficial for the successful acclimatization of *Lycium chinese* var. *Macrophyllum* seedlings (Fig. 7).

3. Discussion

The effectiveness of sterilizing seeds and explant surfaces is influenced by many factors, including the concentration of disinfectants, exposure duration, and the sensitivity of the explant to chemical agents. Achieving a high level of sterilization without compromising seed viability is crucial for subsequent tissue culture. This study confirms that the treatment process of rinsing with running water for 2 hours, soaking in 75% ethanol for 60 seconds, rinsing 3–4 times with sterile water, disinfecting with 1% sodium hypochlorite for 20 minutes, and then rinsing 6–7 times with sterile water is the most effective scheme for balancing sterility and germination rate. For example, in research on peach rootstock systems, using a 20% sodium hypochlorite solution for 15 minutes achieved the best results [19], and for *Camellia yubsienensis* stem explants, treating with 2% sodium hypochlorite for 8 minutes proved to be an effective sterilization method, significantly reducing contamination while maintaining a high survival rate [20]. In blueberry studies, the most effective method was first treating with 75% ethanol for 60 seconds, followed by 4% sodium hypochlorite for 5 minutes [21].

Subsequent experiments focused on the effects of different hormone combinations on callus, root, and shoot induction in young explants. The results showed that the optimal medium for inducing callus was MS supplemented with 6-BA 0.3 mg/L and NAA 1.0 mg/L, achieving an induction rate of 84% in young explants. This aligns with the known function of 6-BA in promoting cell division and differentiation and with previous research showing that young tissues (such as young stem segments) are more sensitive to auxin such as in *Himalayan rice* [22]. However, 6-BA concentration must be carefully optimized as excessive 6-BA may inhibit callus growth or lead to deformed callus. Moreover, different combinations and ratios of hormones significantly affect callus formation in various plants: for instance, in *Psoralea corylifolia* Linn., the combination of 2 mg/L 6-BAP and 0.2 mg/L NAA induces multiple shoots and promotes the accumulation of endogenous putrescine [23]. In *Gardenia jasminoides* Ellis., adding 6-BA and NAA to MS medium promotes the regeneration of adventitious shoots [24], and for *Campanula leblebicii* Yıldırım, petiole and leaf explants were cultured on MS medium containing 0.3 mg/L NAA and 0.5, 1.0, 2.0, or 3.0 mg/L zeatin (ZEA) or 6-benzylaminopurine (BA) [25], and Moosikapala found that 1.0 mg/L 2,4-D significantly affects callus induction in *Garcinia mangostana* [26]. In a study on *Suaeda glauca*, an efficient organogenesis system was developed using 6-Benzylaminopurine (6-BAP) and Indole-3-butyric acid (IBA) [27]. Considering the effectiveness of 6-BA and its widespread use in callus induction, this study selected the optimal concentration to balance efficacy and safety.

The shoot induction results showed that treatment with 0.3 mg/L 6-BA and 0.05 mg/L NAA significantly promoted shoot formation, especially in explants with nodal stems—axillary buds in these segments may contain meristematic tissue that is easily stimulated to develop into shoots under appropriate

cytokinin levels. This is similar to the findings in sweet broad pea [28], where 1.0 mg/L 6-BA in MS medium, or combined with 1.0 mg/L 1-naphthaleneacetic acid (NAA), promoted callus formation and adventitious shoot differentiation. In this study, treatment with 0.8 mg/L 6-BA and 0.05 mg/L NAA (medium C) completely inhibited shoot formation, resulting in a 0% induction rate. Research on the “Tristar” variety of strawberry (*Fragaria × ananassa* Duch.) [29] found that early use of 6-BA promoted stolon formation, while repeated use inhibited stolon growth. This suggests that the inhibitory effect of 6-BA on shoots may be concentration-dependent and time-sensitive. For root induction, 0.2 mg/L NAA effectively induced root primordia formation and root development in *Lycium chinese* tube seedlings, with no significant toxic effects on the plants, making it the optimal concentration for in vitro rooting of this species. For instance, in microshoot culture of peanut (*Arachis hypogaea* L.), NAA effectively induced rooting and aided in successful acclimatization and transplantation [30], and in vitro culture of Masson pine (*Pinus massoniana* Lamb.), NAA combined with other auxins (such as indole-3-acetic acid, IAA) or plant growth retardants (such as paclobutrazol, PBZ) significantly increased rooting rates and root numbers [31–32]. In red raspberry (*Rubus idaeus*), different concentrations and treatment methods of NAA and IBA significantly affected rooting frequency. This phenomenon indicates that NAA is an effective regulator of rooting [33–34].

In summary, this study has established a complete tissue culture system for large-leaf goji. The effective sterilization technique for young stem segments, the suitable combination of growth regulators (PGRs) for callus and bud induction, and the rooting protocol demonstrated in this study provide a feasible approach for protecting this species of significant medicinal and ecological value. The high survival rate during the acclimatization period confirms that this method has the potential for large-scale propagation, contributing to the long-term sustainability of *Lycium chinese* var. *macrophyllum*.

4. Materials and methods

4.1 Plant Materials

Explant materials of *Lycium chinense* var. *Macrophyllum* were collected from the experimental base of Tarim University. They were washed with tap water for 2 hours and then air-dried for later use.

4.2 Culture Medium

Using 1 liter of basic culture medium as an example, its components are 4.74 g of MS powder, 30 g of sucrose, 7.5 g of agar, and 1 liter of purified water, with the pH controlled between 5.8 and 6.2. Typically, the medium is divided into 10 bottles, with each bottle containing 100 ml of medium solution. The medium is then sterilized in an autoclave at 121°C for 20 minutes[10].

4.3 Sterilization of Large-leaved Goji Seedlings

Lycium chinense Mill seedlings were selected as explants and surface sterilized under sterile conditions. Nine disinfection methods were evaluated (Table 1) as follows: ethanol at concentrations of 70% and 75% was applied for 8 minutes, 3 minutes, 0.5 minutes, and 1 minute in sequence, followed by rinsing 3

times with sterile water; subsequently, NaClO at concentrations of 8%, 10%, and 1% was applied for 10 minutes, 15 minutes, and 20 minutes, and finally rinsed 3–7 times with sterile water. The most effective sterilization method identified was used in subsequent experiments. The sterilization rate was calculated using the formula: Sterilization rate = (Number of sterile seeds/Total number of seeds)×100%, Germination rate = (Number of germinated seeds/Total number of seeds)×100%[11].

4.4 Induction of Callus

Stem segments of *Lycium chinense* Mill with a length of 1–2 cm were selected as explants and transferred to MS solid medium containing different concentrations of 6-BA (0, 0.3 mg/L, 0.5 mg/L) and NAA (0.05 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L) to induce callus formation. Culture conditions were set at 25°C with a 12h/12h light/dark photoperiod. Each culture bottle contained 3 leaves or stem segments meeting the requirements, and 10 replicates were set for each hormone concentration combination to ensure data reliability. During the experiment, the timing and growth dynamics of callus formation were carefully recorded, and the induction success rate of callus under different hormone ratios was calculated[12].

Here, “+” indicates callus with a hard texture or root formation and poor growth. “++” indicates callus with a relatively loose texture and relatively good growth. Furthermore, “+++” indicates callus with good growth; “++++” indicates callus with a very loose texture, optimal growth, and highly favorable for subsequent differentiation and culture. The callus induction rate was calculated using the formula: Callus induction rate=(Number of explants producing callus/Total number of explants) × 100%[13].

4.5 Induction of Multiple Shoots

Select well-growing callus tissue and cut it into tissue blocks with a volume of 1 cm³. Transfer them onto MS solid hormone media containing different concentrations of 6-BA (0.3 mg/L, 0.5 mg/L, 0.8 mg/L) and NAA (0.5 mg/L, 0.05 mg/L). The culture conditions are set at a temperature of 25°C with a 12h/12h light/dark cycle. Place 3 callus pieces in each culture bottle, and for each hormone concentration combination, set up 10 replicates to ensure data reliability. During the experiment, carefully record the time of adventitious bud emergence and their growth status, and also count the differentiation of the callus to explore the hormone concentration combination most favorable for differentiation. The formula for calculating the multiple shoot induction rate is: Multiple Shoot Induction Rate=(Number of explants producing multiple shoots/Total number of explants)×100%[14–15].

4.6 Induction of Rooting in Plantlets

Cut the tender stems of well-growing multiple shoots into small segments of 1.5-2.0 cm and transfer them onto MS solid medium supplemented with 0.2 mg/L NAA. Cultivate them under conditions of 25°C and a 12h/12h light/dark cycle, and observe their rooting situation[16].

4.7 Acclimatization and Transplanting

When the tissue-cultured seedlings reach a height of 5 cm and develop 3–5 roots, close the culture bottles and gradually move them to the natural environment for acclimatization, which lasts for 7 days. Remove the seedlings from the bottles and carefully wash off the adhering medium from their roots with warm water not exceeding 35°C. Then, transplant the seedlings into nutrient pots or flower pots filled with a substrate of imported Peat and Vermiculite mixed in a 3:1 ratio. After transplanting, place the seedlings in an intelligent greenhouse with relative humidity controlled at 65% and temperature at 25°C for acclimatization cultivation[17–18].

4.8 Statistical analysis

Tests of statistical significance was performed using one-way ANOVA with post hoc analysis. Differences were considered significant if the p-value was < 0.05.

5 Conclusion

This study established an efficient tissue culture regeneration system for *Lycium chinense* Mill..The optimal sterilization procedure for explants is as follows: rinse under running water for 2 hours, soak in 75% ethanol for 60 seconds, rinse 3–4 times with sterile water, disinfect with 1% sodium hypochlorite for 20 minutes, and rinse 6–7 times with sterile water. This procedure has a low contamination rate and does not affect explant viability. The best medium for callus induction is MS supplemented with 6-BA 0.4 mg/L and NAA 1.2 mg/L, achieving an induction rate of 84% and low browning. The optimal combination for bud differentiation is MS supplemented with 6-BA 0.3 mg/L and NAA 0.05 mg/L, with an induction rate of 80%. Rooting is most effective in MS medium with NAA 0.2 mg/L, achieving a rooting rate of 85%. Acclimatization using a substrate mixed with imported Pindstrup peat and vermiculite in a 3:1 ratio resulted in a transplant survival rate of 95%. This system provides reliable technical support for the preservation of large-leaf goji germplasm resources, rapid propagation, and genetic improvement.

Declarations

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Author Contribution

Hemeng Wang conceived and designed the experiments, as well as provide experimental platforms and funding. Guanghui Wei and Jiayue Zhu performed the experiments, Shijing Wei and Buayi Shamu analyzed the data and drafted the manuscript. Hemeng Wang, Conghao Zhang and Ye Yao revised the manuscript. All authors read and approved the final manuscript.

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Figures

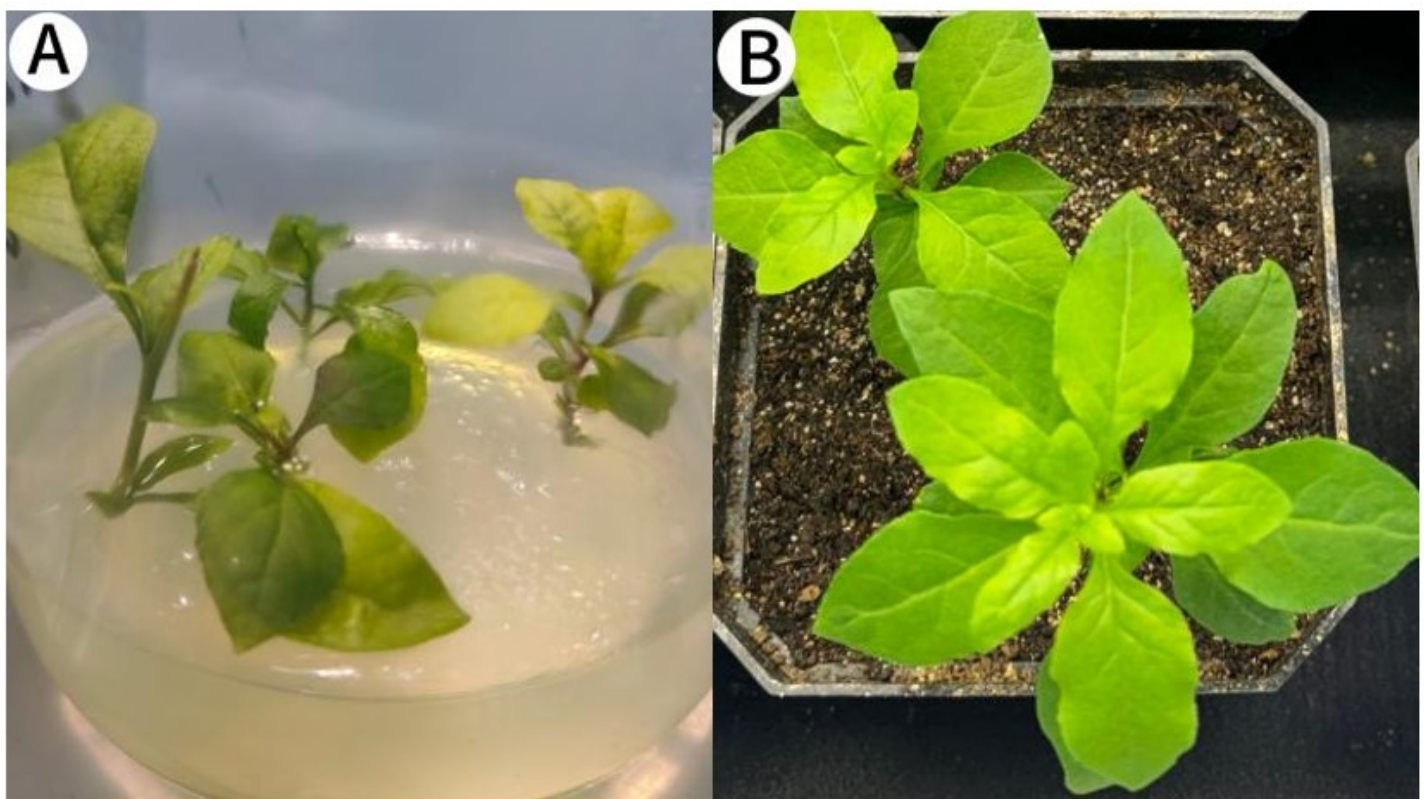


Figure 1

Effect of different disinfection methods on *Lycium chinense* var. *macrophyllum*

(A) Treatment group 9 (best effect); (B) *Lycium chinense* var. *macrophyllum* seedlings

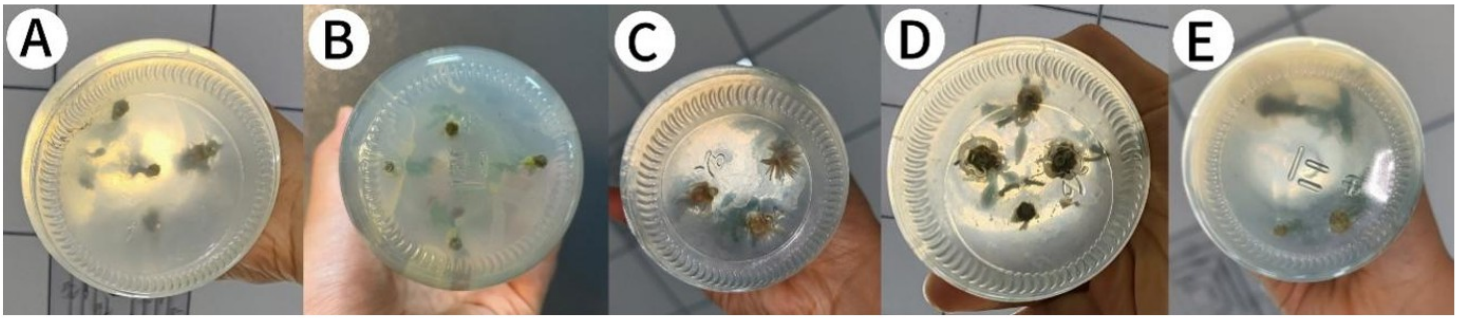


Figure 2

The effect of different ratios of NAA and 6-BA on the induction of callus in *Lycium chinense* var. *macrophyllum*

(A) MS medium supplemented with 0.3 mg/L 6-BA and 0.05 mg/L NAA; (B) MS medium supplemented with 0.5 mg/L 6-BA and 0.5 mg/L NAA; (C) MS medium supplemented with 0.25 mg/L NAA; (D) MS medium supplemented with 0.3 mg/L 6-BA and 1 mg/L NAA; (E) MS medium supplemented with 1 mg/L 6-BA and 0.2 mg/L NAA.



Figure 3

The extent of callus induction in *Lycium chinense* var. *macrophyllum* using culture medium with 0.3 mg/L NAA and 1 mg/L 6-BA (after 4 weeks of culture)

Note: "-" indicates no callus formation, "+" indicates a small amount of callus, slow growth, and dense texture, then "+++" indicates healthy-colored callus, loose texture, and vigorous growth

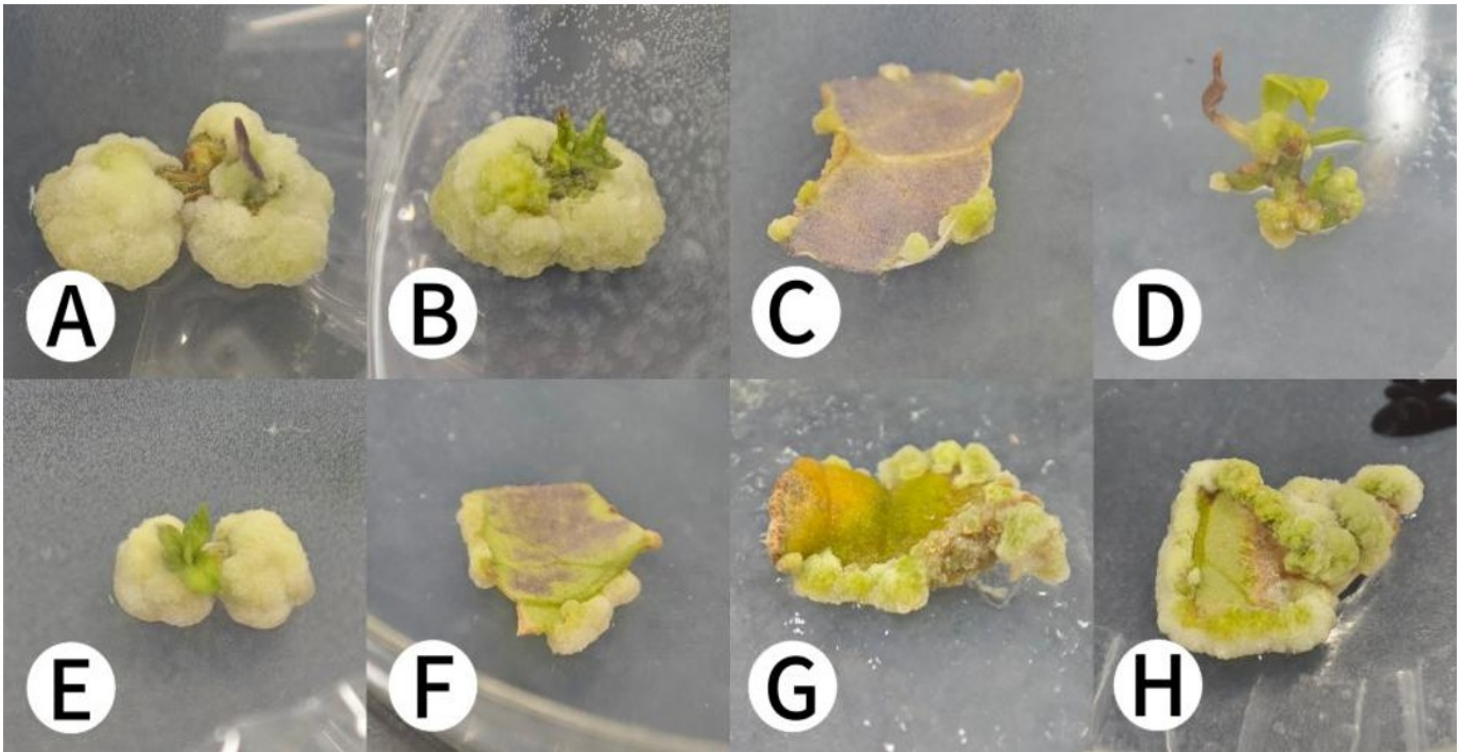


Figure 4

Effects of different concentration gradients of NAA and 6-BA on callus induction of *Lycium chinense* var. *macrophyllum* after 3 weeks of culture.

(A) MS medium supplemented with 0.2 mg/L 6-BA and 0.8 mg/L NAA; (B) MS medium supplemented with 0.2 mg/L 6-BA and 1.0 mg/L NAA; (C) MS medium supplemented with 0.2 mg/L 6-BA and 1.2 mg/L NAA; (D) MS medium supplemented with 0.3 mg/L 6-BA and 0.8 mg/L NAA; (E) MS medium supplemented with 0.3 mg/L 6-BA and 1.2 mg/L NAA; (F) MS medium supplemented with 0.4 mg/L 6-BA and 0.8 mg/L NAA; (G) MS medium supplemented with 0.4 mg/L 6-BA and 1.0 mg/L NAA; (H) MS medium supplemented with 0.4 mg/L 6-BA and 1.2 mg/L NAA.

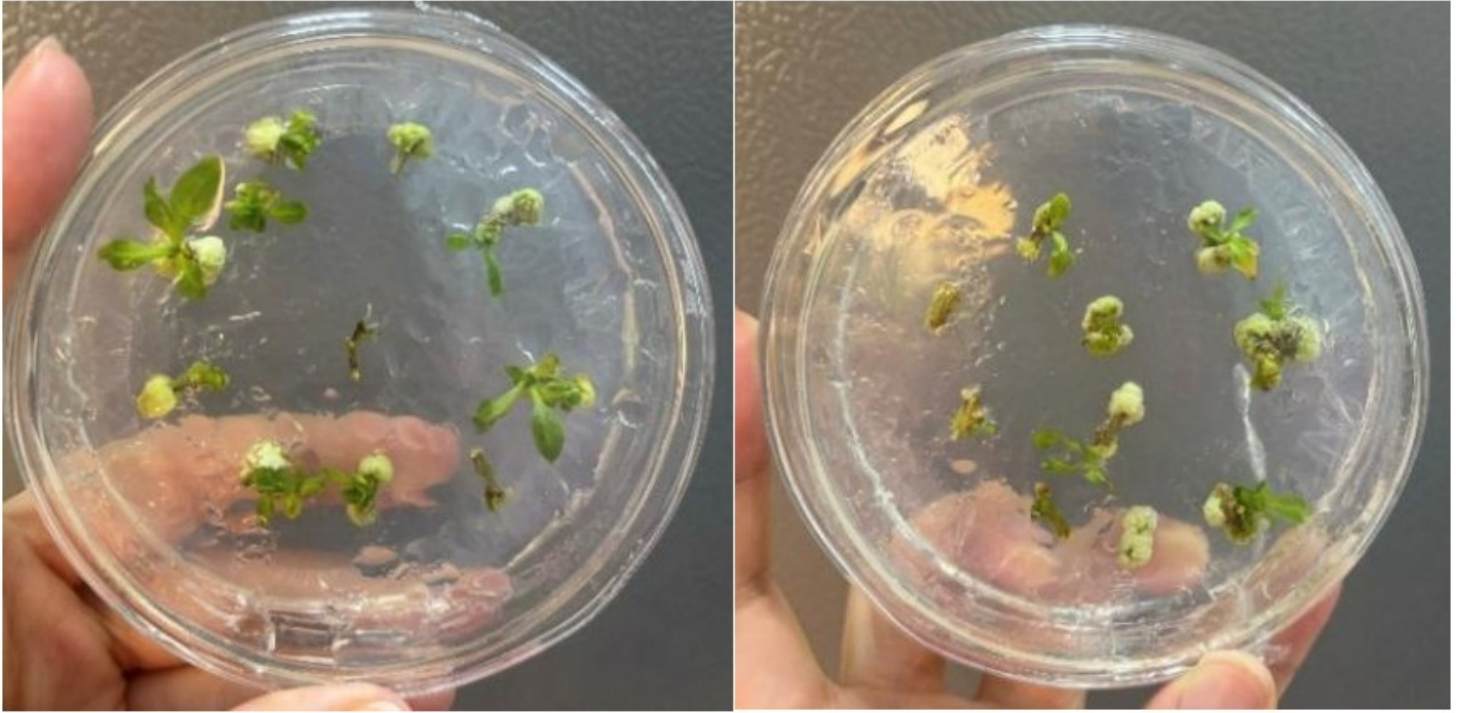


Figure 5

Effect of 0.05 mg/L NAA and 0.3 mg/L 6-BA hormone medium on bud induction of large-leaf goji stem segments (after 5 weeks of culture)



Figure 6

Phenotype of rooting induction of *Lycium chinense* var. *macrophyllum* on 0.2 mg/L NAA hormone medium (after 5 weeks of culture)

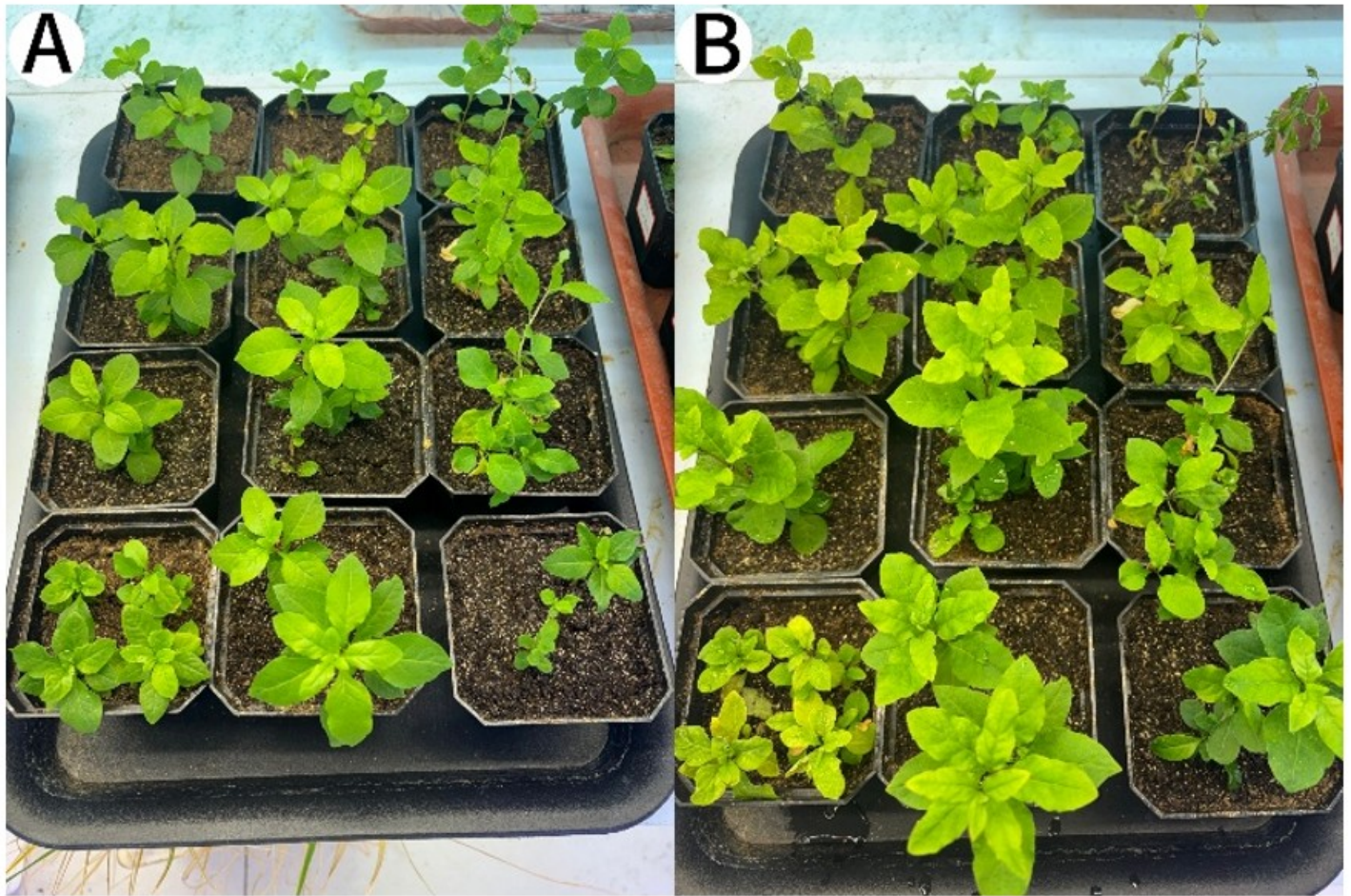


Figure 7

Acclimatization of *Lycium chinevar. Macrophyllumon* seedlings

(A) Seedlings with well-developed root systems are planted in small plastic pots containing transplant substrate On the first day of acclimatization; (B) Transplanted in sterile soil for 2 weeks