*Pak. J. Bot.*, 57(5): DOI: http://dx.doi.org/10.30848/PJB2025-5(16)

# ESTABLISHMENT OF TISSUE CULTURE REGENERATION SYSTEM OF *GYMNOTHECA CHINENSIS* DECNE.

**WAN-YUN WU†, SHU-PING WU† AND ZHAN-NAN YANG***

[1]*Key Laboratory for Information System of Mountainous Areas and Protection of Ecological Environment of Guizhou Province, Guizhou Normal University, Guiyang 550025 Guizhou, China*
**Corresponding author's e-mail: yangzhannan@163.com*
†*Authors contributed equally to this work*

**Abstract**

In order to establish a simple and efficient tissue culture regeneration system for *Gymnotheca chinensis* Decne., the tender stems and terminal buds of *G. chinensis* cultivated in the wild as experimental materials to explore the optimal explant, sterilization conditions of explant, medium for adventitious bud induction, and medium for rooting and subculture in the process of plant tissue culture. The experimental results showed that the top bud as the explant was sterilized by using 0.5 mg/L of sodium penicillin and streptomycin for 3 h+75% ethanol for 30 s+0.1%$HgCl_2$ for 20 min+0.1%$HgCl_2$ for 15 min, with the pollution rate of 22.2% and the browning rate of 33.3%. MS+2 mg/L 6-BA+0.5 mg/L NAA+0.2 g/L activated carbon was the best primary medium with 96.7% bud, MS+1mg/L NAA+0.5 mg/L IBA+0.2 g/L activated carbon as rooting medium, the rooting rate was 66.7%. The best secondary medium was MS+1.0 mg/L NAA+1.0 mg/L IBA+0.2g/L activated carbon with 88.9%, and the survival rate of tissue seedlings reached 100% after transplanting to humus soil 5 d after domestication. This stable tissue culture and regeneration system of *G. chinensis*, which will provide technical reference for artificial breeding and biological experiments of *G. chinensis*.

**Key words:** *Gymnotheca chinensis*, Tissue culture, Terminal bud, Regeneration system.

## Introduction

*Gymnotheca chinensis* Decne. is a herb, also known as Shuibaibu Redead grass (Xiao *et al.*, 2016). *Gymnotheca* is a unique genus in China (Xiao *et al.*, 2016), mainly distributed in Guangxi, Yunnan, Guizhou and Sichuan and other southwest regions, and is commonly used as a folk Chinese herbal medicine in the southwest of China (Chen *et al.*, 2018). In the "*Chinese traditional medicine resources Zhiyao*" records *G. chinensis* whole grass into medicine, taste pungent, sexual temperature (Xiao *et al.*, 2016), internal use with the treatment of lung deficiency cough, urinary drenching pain, edema, etc.(Zhang *et al.*, 2016), external use can be used to treat bruises, centipede bites and so on (Xiao *et al.*, 2016). *G. chinensis* is rich in lignin and alkaloids. In recent years, a series of structurally novel lignin and alkaloid components have been isolated from the nude capsule, with anti-tumour, anti-virus, hepatoprotective, anti-platelet aggregation and other biological activities (Chen *et al.*, 2018). In addition, it can also be used as an ornamental plant and tea, therefore, *G. chinensis* has great prospects for development in medical and ornamental aspects.

*G. chinensis* breeding process, growth to the late stage prone to disease (Zhao *et al.*, 2022). The most common diseases are leaf blight; leaf blight will lead to nude capsule rhizomes becoming brown and rotting. Serious cases may lead to the death of the leaves and the complete rotting of the rhizomes (Jin *et al.*, 2015) . May change with the weather and the growth of plants, cause the accumulation of bacteria, resulting in the emergence of leaf blight (Perelló *et al.*, 2024). Most of the treatments choose spraying drugs for prevention and control, but drug prevention and control will produce serious pollution to the environment (Hassaan & Nemr, 2020). Tissue culture technology requires strict sterilization of the explants, which can prevent microorganisms from affecting cell differentiation and reduce the accumulation of pathogens (Mitter *et al.*, 2019), and may therefore be a good way to improve the problem of *G. chinensis* diseases. Plant tissue culture technology also has the advantages of a short reproduction cycle, low cost, and the ability to avoid pesticide and heavy metal pollution (Akila & Jayeni, 2023). Currently, this technology is widely used in the field of medicinal plants, which can improve the quality and yield of secondary metabolites of medicinal plants (Danova & Pistelli, 2022), and plays an important role in resource protection and sustainable utilization (Anushi *et al.*, 2023).

There are also certain challenges associated with plant tissue culture techniques, with contamination, browning, and vitrification being the three major problems of tissue culture during cultivation (Liu *et al.*, 2023). The way in which explants are sterilized and disinfected is difficult to control, especially for plants that are rich in endophytes, the process of sterilization and disinfection, as well as the choice of explant, is more challenging. Excessive disinfection causes browning of explants (Ahmadpoor *et al.*, 2022)vitrification (Tung *et al.*, 2021)and etiolation (Zhang *et al.*, 2022). Lighter disinfection easily to causes contamination (Wang *et al.*, 2019), and it is difficult to carry out standardized production. There are certain techniques for *Sterilizing* exosomes; the concentration and type of disinfectant, as well as the duration of disinfection, are lower for exosomes containing fewer bacteria, and vice versa for exosomes that are richer in bacteria. Ye *et al*., (2022) used seeds of *Cichlidium fischeri* as exosomes, and the seeds, as the endpoint of vertical transmission of endophytes, contained relatively low diversity of endophytes Disinfection with 'ethanol+$HgCl_2$' can obtain low contamination sterile seedlings. While Golovatskaya *et al*., (2024) used the underground stems of *Cichlidium fischeri* as the explant, which has a strong mutual activity with soil microorganisms (Kandel *et al.*, 2017), the sterilization method utilised a mixture of 'sodium penicillin and streptomycin solution for soaking+$HgCl_2$' composite sterilization. Seeds, leaves, petioles, stems and buds, of plants are generally used as explants for plant tissue culture (Tam *et al.*, 2021). Lin *et al*., (2022) demonstrated that the ecological niche of the plant affected the microbial content in the plant, and that the general

microbial content roots>stems>leaves. Aghaali *et al*., (2019) utilized different types of poppy explants for culture and found that the overall results of leaves as explants were the best. Deng *et al*., (2023) selected the leaves, stem tips, petioles and underground stem segments of *Magnolia* explants, and the results showed that the survival rate of leaves, stem tips and petioles was significantly higher than that of underground stems. Selected exosomes and sterilization facilitate research in plant tissue culture techniques.

Tissue culture studies on *G. chinensis* have not been reported so far, and it is of great significance to select suitable exosomes and establish a stable and efficient *G. chinensis* rapid propagation system with a low contamination rate to guide the *In vitro* cultivation of endophyte-containing exosomes as well as the production of medicinal plants. The present study employed gymnocarpus and apex buds as explants. Single-factor and orthogonal tests were conducted to determine suitable disinfection methods and growth conditions for gymnocarpus. Using MS as the basic medium and adding different growth regulators, a stable and efficient regeneration system was established by counting contamination, sprouting and rooting. This study establishes the tissue culture and regeneration system of *G. chinensis*, aiming to provide new technical parameters and theoretical basis for the cultivation and exploitation of *G. chinensis*.

## Material and Methods

**Experimental material:** The experimental materials were collected in April 2023 from Wudang District, Guiyang City, Guizhou Province (26°63'N, 106°75'E). Plants with thick stems, free of pests and diseases and healthy growth were dug and brought back to the laboratory to be cultivated in pots and used as back-up materials at a later stage. Young stems and terminal buds of luxuriantly growing *G. chinensis* were selected as explants. The basic medium for bud induction and rooting induction was MS, sucrose 15 g/L, agar 10 g/L, activated carbon powder 2g/L, pH value of the medium was 5.8~6.2, and different concentrations of NAA and 6-BA were added as the primary medium. Different concentrations of NAA, IAA and IBA were added to root medium for root induction. Subculture medium was added with different concentrations of NAA and IBA as subculture. The prepared medium was packed in 200 mL group culture bottles, each containing 50 mL, and autoclaved at 121℃ for 20 min under the following conditions: temperature (25±2)℃, light 1500~2000 lx, and light 12 h per day.

**Sterilization and inoculation of the explants:** At first, the young stems of *G. chinensis* were selected as explants, and the explants were first rinsed with running water and soaked for 12 h. After that, they were transferred to the aseptic operating table to be soaked with 0.5 g/L penicillin and streptomycin mixed solution for different times (set up 3 treatments at 0, 0.5, and 1 h), and then washed with sterile water washing 4~5 times, and then soaked in 75% alcohol for 30 s, and washed with sterile water for 3 times, and then transferred to 0.1% $HgCl_2$ solution for disinfection (set up 4 treatments of 0, 5, 10, 15, 20 min), and washed 4~5 times with sterile water, combining a total of 15 treatments (Table 1). Sterilization and clean-up were done with strong shaking to ensure for the subject to be in full contact with the solution. The explants were cut into lengths of about 1.5 cm and then inserted into the primary medium. Five parallels were set up for each sterilization treatment, and each vial was inoculated with four exosomes and replicated three times. The contamination rate and browning rate of the exosomes were recorded and counted at 20 days.

**Screening of explants and optimization of disinfection methods:** The results of the initial sterilization treatment revealed high levels of contamination, which subsequently manifested approximately one week later. This observation suggests that the *G. chinensis* contained a substantial abundance of endophytes. In the case of plants with a high endophyte population, a two-step sterilization operation may be employed (Chen *et al.*, 2018). In order to reduce the contamination rate, the young stems and terminal buds with good growth were selected as explants, while the soaking time of the mixed solution of penicillin and streptomycin was lengthened (set up 1, 3, 6 h 3 treatments), Soak in 75% alcohol for 30 s and then sterilized twice with 0.1% $HgCl_2$ solution, the first $HgCl_2$ solution was sterilized (set up 15, 20 min 2 treatments), and a second $HgCl_2$ solution sterilization was carried out (set up 10, 15, 20 min 3 treatments), a total of 18 groups were combined (Table 2), and each disinfectant was washed with sterile water 4~5 times after disinfection. Five parallels were set up for each sterilization treatment, and each vial was inoculated with four exosomes and replicated three times. The contamination rate and browning rate of the exosomes were recorded and counted at 20 days.

**Induction of adventitious shoots:** The induction of adventitious shoots was carried out by using terminal buds as explants and MS as the basic medium with the addition of different concentrations of NAA and 6-BA, setting up NAA (set up 0, 0.2, 0.5, 1, 1.5, 2 mg/L 6 treatments) and 6-BA (set up 0, 0.5, 1, 1.5, and 2 mg/L 5 treatments) in a total of 9 combinations (Table 6). The optimal successional medium was selected. Three parallels were set for each group of treatments, and each parallel was connected with 5 vials, and each vial was inoculated with 4~5 explants. The number and rate of germination of the explants were recorded and counted at 25 days, and the best primary medium was selected.

**Induction of taking root:** Seedlings grown in the optimal starter medium for up to 30 days with good growth were transferred to rooting medium, and the oxidised portion of the original insertion into the starter medium was cut before transfer. Rooting medium was screened by using MS as the basic medium with the addition of different concentrations of NAA, IAA, and IBA, and the combinations of NAA (0.0, 0.5, and 1 mg/L 3 treatments) and IAA (0.5 and 1 mg/L 2 treatments) and IBA (0.5 and 1 mg/L 2 treatments) were set up, with a total of 10 groups of combinations (Table 3). Three parallels were set up for each group of treatments, and each parallel was knotted with 5 vials, and each vial was inoculated with 1 explant. The number of rooted seedlings, rooting rate, average number of roots, and average root length of the explants were recorded and counted at 40 d. The number of rooted seedlings, rooting rate, average number of roots, and average root length were recorded and counted at 40 days.

**Table 1. different disinfection methods of stems as explants.**

| Experi-mental treatments | Soaking time of penicillin and streptomycin mixed solution immersion time | Sterilization duration of 75% alcohol | Sterilization duration of 0.1% $HgCl_2$ |
|---|---|---|---|
| S1 | - | 30 | - |
| S2 | - | 30 | 5 |
| S3 | - | 30 | 10 |
| S4 | - | 30 | 15 |
| S5 | - | 30 | 20 |
| S6 | 0.5 | 30 | - |
| S7 | 0.5 | 30 | 5 |
| S8 | 0.5 | 30 | 10 |
| S9 | 0.5 | 30 | 15 |
| S10 | 0.5 | 30 | 20 |
| S11 | 1 | 30 | - |
| S12 | 1 | 30 | 5 |
| S13 | 1 | 30 | 10 |
| S14 | 1 | 30 | 15 |
| S15 | 1 | 30 | 20 |

Note: - Indicates not progress or addition

**Table 2. optimization of disinfection treatment of different explants.**

| Experi-mental treatments | Soaking time of penicillin and streptomycin mixed solution immersion time (h) | Sterilization duration of 75% alcohol (s) | Sterilization duration of 0.1% $HgCl_2$ | |
|---|---|---|---|---|
| | | | Step One | Step Two |
| A1 | 1 | 30 | 15 | 10 |
| A2 | 1 | 30 | 15 | 15 |
| A3 | 1 | 30 | 15 | 20 |
| A4 | 1 | 30 | 20 | 10 |
| A5 | 1 | 30 | 20 | 15 |
| A6 | 1 | 30 | 20 | 20 |
| A7 | 3 | 30 | 15 | 10 |
| A8 | 3 | 30 | 15 | 15 |
| A9 | 3 | 30 | 15 | 20 |
| A10 | 3 | 30 | 20 | 10 |
| A11 | 3 | 30 | 20 | 15 |
| A12 | 3 | 30 | 20 | 20 |
| A13 | 6 | 30 | 15 | 10 |
| A14 | 6 | 30 | 15 | 15 |
| A15 | 6 | 30 | 15 | 20 |
| A16 | 6 | 30 | 20 | 10 |
| A17 | 6 | 30 | 20 | 15 |
| A18 | 6 | 30 | 20 | 20 |

Note: -Indicates not progress or addition

**Table 3. primary induction of terminal buds by different hormone combinations.**

| Experimental treatments | Concentration of NAA (mg/L) | Concentration of IAA (mg/L) | Concentration of IBA (mg/L) |
|---|---|---|---|
| C1 | 0.5 | 0.5 | - |
| C2 | 1 | 0.5 | - |
| C3 | 0.5 | 1 | - |
| C4 | 0.0 | 1 | |
| C5 | 0.5 | - | 0.5 |
| C6 | 0.5 | - | 1 |
| C7 | 1 | - | 0.5 |
| C8 | 0.0 | - | 1 |

Note: - Indicates not progress or addition

**Screening of subculture medium:** The optimal rooting medium was preliminarily determined, and the optimal subculture medium was screened based on this. In the rooting medium, healthy naked capsule seedlings were grown, and the buds were transferred to the subculture medium. Selection of medium with better rooting effects by treatments for initial root induction. On this basis, the concentration of different NAA and IBA was adjusted to screen out the best subculture medium. NAA (0.5 and 1.0 mg/L 2 treatments) and IBA (0.8, 1.0 and 1.5 mg/L 3 treatments) were set up in a combination of 6 groups (Table 8). Each treatment was set up in 3 parallels, and each parallel was inoculated with 5 vials, 1 explant per vial. The

number of rooted seedlings, rooting rate, average rooting number, and average root length of the explants were recorded and counted at 40 days.

Seedling refining and transplanting: Well-grown sterile seedlings with a well-developed root system in successional culture were selected for transplanting experiments. In order to simulate the nursery soil after fine cultivation, the culture substrates of three different soils were set up: E1 all calcareous loess; E2 calcareous loess: humus = 1:1; E3 all humus. Both types of soil were naturally air-dried, milled through a 10-mesh sieve, and filled into pots of 7.5 cm × 4.5 cm × 12 cm (top-bottom radius × bottom-bottom radius × height) size, with 1 kg of soil in each pot. The transplanted *G. chinensis* seedlings were first slowly uncovered at the appropriate temperature for 5 d, sprayed with water once a day in the morning, noon and night, so that the seedlings could experience humidity, and washed with water to clean the medium attached to them when transplanted, avoiding direct sunlight, and sprayed with water in the morning and evening in the first week after transplanting. The survival rate and the growth condition of the seedlings were then counted after 45 d. The seedlings were then transplanted in the same pots for 5 times each treatment. Each treatment was replicated in 5 pots, and one plant was planted in each pot.

**Statistical analysis of data:** The collected data were analysed and counted using Excel 2016 and SPSS 20.0 statistical software, and significant differences were analysed between the different treatments using ANOVA and LSD methods, and significant differences were considered to exist at $p<0.05$.

$$\text{Contamination rate (\%)} = \frac{\text{Number of contaminated exosomes}}{\text{Number of exosomes inoculated for that treatment}} \times 100$$

$$\text{Browning rate (\%)} = \frac{\text{Number of browned exosomes}}{\text{Number of inoculated exosomes for that treatment}} \times 100$$

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seedlings}}{\text{Number of total seedlings inoculated in that treatment}} \times 100$$

$$\text{Rooting rate (\%)} = \frac{\text{Number of rooted seedlings}}{\text{Number of total seedlings inoculated in that treatment}} \times 100$$

**Table 4. The effect of different disinfection methods on naked capsule stem segment.**

| Experimental treatments | Inoculation number | Contamination rate (%) | Browning rate (%) | Browning degree |
|---|---|---|---|---|
| S1 | 60 | 100 ± 0.0a | 0.0 ± 0.0f | - |
| S2 | 60 | 100 ± 0.0a | 0.0 ± 0.0f | - |
| S3 | 60 | 93.3 ± 5.8a | 0.0 ± 0.0f | - |
| S4 | 60 | 93.3 ± 11.5a | 8.3 ± 2.9e | + |
| S5 | 60 | 83.33 ± 12.6abc | 13.3 ± 2.9cde | + |
| S6 | 60 | 98.3 ± 2.9a | 0.0 ± 0.0f | - |
| S7 | 60 | 95.0 ± 5.0a | 0.0 ± 0.0f | - |
| S8 | 60 | 86.7 ± 5.8abc | 13.3 ± 2.9cde | + |
| S9 | 60 | 83.3 ± 12.6abc | 16.7 ± 2.9bcd | + |
| S10 | 60 | 73.3 ± 15.3bc | 21.7 ± 5.8ab | ++ |
| S11 | 60 | 96.7 ± 5.8a | 8.3 ± 5.8e | + |
| S12 | 60 | 91.7 ± 2.9ab | 10.0 ± 8.7de | + |
| S13 | 60 | 86.7 ± 5.8abc | 20.0 ± 5.0abc | ++ |
| S14 | 60 | 73.3 ± 15.3bc | 25.0 ± 5.0a | ++ |
| S15 | 60 | 71.7 ± 20.2c | 21.7 ± 5.8ab | ++ |

Notes: + The more shows the more serious the degree of browning; - Indicates no corresponding symptoms; Different lowercase letters in the same column indicate significant difference among the treatments at 0.05 level

## Results

**Sterilization of *G. chinensis* explants by different sterilization methods:** Sterilization of explants is a critical step in the success of plant tissue culture, and different sterilization treatments for naked explants reduce the rate of contamination of naked explants to a certain extent.

**Sterilization effect of the primary disinfection method on the explants:** It is critical for the exosome sterilization to obtain sterile seedlings, and the choice of sterilizing agent and time of sterilization is very important (Bhadane & Patil, 2016). The results from Table 4 showed that the sterilization effect of using 75% ethanol alone (S1) and using 75% ethanol, 0.5 mg/L penicillin+0.5 mg/L streptomycin mixed solution in combination (S6, S11) and 75% ethanol, 0.1% $HgCl_2$ in combination was not as good as the sterilization effect of the penicillin + streptomycin mixed solution, 75% ethanol, 0.1% $HgCl_2$ in combination of three agents. The contamination rate was not significantly different between the single reagent and the two reagents, indicating that the combination of the three reagents was the best choice. The treatment time of penicillin + streptomycin mixed solution was constant, and the treatment time of 0.1 % $HgCl_2$ was prolonged. The pollution rate was decreased with the increase of treatment time, but the browning rate increased with the increase of treatment time.

When the penicillin + streptomycin mixture and 0.1% $HgCl_2$ treatments were prolonged at the same time, the contamination rate was lower in treatment S15 relative to the other groups, with a contamination rate of 71.7%. Despite undergoing different sterilization treatments, the rate of contamination was relatively high, mainly due to fungal growth observed around the explants at around 7 days of inoculation (Fig. 1A & B) and bacterial growth around the explants at around 15 days of inoculation (Fig. 1C & D). Titov, *et al* confirmed that microbial contamination occurring 7 days after inoculation was probably caused by endophytic bacteria (Titov *et al.*, 2007).

**The sterilization efficacy of a two-step sterilization method on various explants:** Two-step sterilization is usually used for explants containing abundant endophytic bacteria (Wen *et al.*, 2024). As can be seen from the results in Table 5, the contamination rate of exosomes in the two-step sterilization method was significantly reduced, indicating that the two-step sterilization method was effective in controlling the growth of endophytes in plants. It was also found that the contamination rate of apical buds was overall lower than that of stems, and that the microbial content of plants was affected by the ecological niche of the plant, with the apical part of the plant containing fewer microorganisms than the other parts of the plant. The soaking time of 0.5 mg/L penicillin+0.5 mg/L streptomycin mixture was 1, 3 and 6 h. The contamination rate gradually decreased with the increase of soaking time, but the browning was quite serious at 6 h, and the browning rate of stems as explants was as high as 68.9% (A17). The terminal bud was an explant, and the Browning rate was significantly increased to 94.4% (A18). The first step of 0.1% HgCl2 disinfection was set for two times, and the results showed that the contamination rate was significantly higher after 20 min of treatment than after 15 min of treatment (treatment A7~A18). In the second step of 0.1% $HgCl_2$ disinfection, the contamination rate was higher for 10 min of disinfection and the browning rate was relatively high for 20 min of disinfection. Among the different treatments, the lowest contamination rate was 27.8% for stems as explants (A17), and the contamination rate was significantly reduced to 8.9% for terminal buds as explants (A18), but browning rates were high in both sets of treatments. Combined with the aforementioned factors, in the two-step sterilization method, the contamination rate of A11 top bud as an explant was recorded at 22.2%, while the Browning rate reached 33.3%, indicating optimal efficacy. The most effective sterilization outcome was achieved by subjecting the explants to a sequential treatment involving soaking in a 0.5 mg/L antibiotic solution for 3 hours, followed by exposure to 75% ethanol for 30 seconds, and finally treating them with 0.1% $HgCl_2$ for 20 minutes and an additional 15 minutes.

**Screening of primary culture medium:** Different combinations of NAA and 6-BA were used for the induction of nude capsule buds, and the different hormone combinations were cultured until 25 d to observe the effect of different hormone combinations on the germination rate of nude capsule. The results showed that, without any growth hormone in the medium, the germination rate was very low, only 16.7% (B0), and there was a significant difference in the germination rate compared with other groups (Table 6). The combination of NAA and 6-BA resulted in a significant increase in germination rate. When the concentration of NAA was kept constant at 0.2 mg/L, the germination rate was increased with the gradual increase of 6-BA concentration, and the germination rate reached 91.7% when the concentration of 6-BA was increased to 2 mg/L (B4). When the concentration of NAA was increased, the germination rate showed a tendency of increasing and then decreasing, in which the germination rate of B6, B7 and B8 decreased with the increase of NAA concentration, and there was no significant difference in the germination rate of the three groups. The combination of NAA and 6-BA at appropriate concentration was chosen to favour the germination effect of nude capsule, and the highest germination rate of nude capsule was achieved in treatment B5, with the germination rate as high as 96.7%, and the plants grew in a healthy potential. Finally, MS+6-BA 2 mg/L+NAA 0.5 mg/L was the best medium for primary induction.

**Screening of rooting medium:** Different combinations of hormones were designed to induce rooting in naked capsules. Several hormone combinations were not very effective in rooting, and it took a long time to induce rooting, with the fastest rooting taking nearly 25 days before adventitious roots started to grow. The specific rooting of naked capsule is shown in Table 7 and Fig. 2. Different hormone combinations showed significant differences in the average number of roots and average root length of naked capsule. When one hormone (C4, C8) was used alone, the rooting effect was very poor, and the rooting rate of C8 was as low as 0%. Rooting was also poor when NAA and IAA were used in combination, with the highest rooting rate reaching only 20% (C3) at a concentration of 0.5 mg/L for NAA and 1 mg/L for IAA but showing a significant increase in rooting compared to C1 and C2; treatment C3 corresponds to Fig. C in Fig. 2, where the roots are sparse and short. Roots under the C4 treatment were milky white in colour and gradually decayed after a period of time (Fig. 2D). In the other treatments, due to the lack of rooting, the seedlings were thin, and the leaves began to wilt and yellow, and growth was retarded. The combination of different concentrations of NAA and IBA resulted in the highest rooting rate of 66.7% (C7), and the average number of roots and root length were also the best among the eight different treatments, with 19.3 roots and 4.8 cm, respectively, and the overall showed significant differences compared with the other groups; as can be seen from Figure G in Figure 2, the foliage of the naked capsule under the treatment of C7 was more fertile, and the plant was growing in a healthier way. In the C5 treatment (Fig. 2E), there was no rooting, but there was a sign of proliferation, and the rooting rate was increased by 33.3% with the increase of IBA concentration at the same concentration of NAA (C6), while the rooting rate was increased to 66.7% with the increase of NAA concentration at the same concentration of IBA, and the rooting was thicker and stronger in the C6 treatment (Fig. 2F), but not as dense and long as that in the C7 treatment. Therefore, 1mg/L NAA+0.5mg/L IBA was the best medium for the initial induction of rooting in naked capsules.

**Table 5. The effect of two-step sterilization on different explants.**

| Experimental treatments | Contamination rate (%) | | Browning rate (%) | | Browning degree | |
|---|---|---|---|---|---|---|
| | Stem | Terminal bud | Stem | Terminal bud | Stem | Terminal bud |
| A1 | 100.0 ± 0.0a | 58.9 ± 13.5a | 0.0 ± 0.0g | 5.6 ± 9.6i | - | + |
| A2 | 91.1 ± 10.2ab | 44.4 ± 13.9ab | 8.9 ± 8.4fg | 16.7 ± 3.3ghi | + | + |
| A3 | 83.3 ± 3.4b | 48.9 ± 3.8ab | 11.1 ± 5.1fg | 8.9 ± 7.7hi | + | + |
| A4 | 83.3 ± 3.4b | 46.7 ± 0.0ab | 8.9 ± 5.1fg | 20.0 ± 6.7ghi | + | + |
| A5 | 77.8 ± 15.0bc | 55.6 ± 9.6a | 16.7 ± 0.0ef | 16.7 ± 3.3ghi | + | + |
| A6 | 76.7 ± 8.8bc | 50.0 ± 0.0ab | 22.2 ± 11.7ef | 27.8 ± 9.6fgh | + | ++ |
| A7 | 66.7 ± 14.5cd | 48.9 ± 1.9ab | 27.8 ± 5.1de | 17.8 ± 1.9ghi | ++ | + |
| A8 | 58.9 ± 1.9de | 47.8 ± 13.9ab | 22.2 ± 11.7ef | 21.1 ± 8.4ghi | + | ++ |
| A9 | 55.6 ± 13.9def | 48.9 ± 1.9ab | 31.1 ± 7.0cde | 27.8 ± 9.6fgh | ++ | ++ |
| A10 | 48.9 ± 1.9efg | 33.3 ± 0.0bc | 38.9 ± 9.6cd | 31.1 ± 3.8efg | ++ | +++ |
| A11 | 40.0 ± 11.5fgh | 22.2 ± 10.7cd | 38.9 ± 9.6cd | 33.3 ± 0.0efg | ++ | ++ |
| A12 | 33.3 ± 6.7gh | 16.7 ± 14.5cd | 44.4 ± 12.6bc | 43.3 ± 13.3def | ++ | +++ |
| A13 | 40.0 ± 11.5fgh | 14.4 ± 12.6d | 55.5 ± 3.9ab | 50.0 ± 14.5cde | +++ | +++ |
| A14 | 41.1 ± 8.4fgh | 22.2 ± 5.1cd | 58.9 ± 7.0ab | 61.1 ± 19.2bcd | +++ | ++++ |
| A15 | 33.3 ± 6.7gh | 16.7 ± 14.5cd | 61.1 ± 19.2a | 66.7 ± 16.7bc | +++ | ++++ |
| A16 | 28.9 ± 5.1h | 25.6 ± 8.4cd | 66.7 ± 0.0a | 77.8 ± 25.5ab | ++++ | ++++ |
| A17 | 27.8 ± 5.1h | 16.7 ± 14.5cd | 68.9 ± 3.8a | 87.8 ± 10.7a | ++++ | +++++ |
| A18 | 31.1 ± 1.9h | 8.9 ± 7.7d | 66.7 ± 0.0a | 94.4 ± 9.6a | ++++ | +++++ |

Notes: + The more shows the more serious the degree of browning; - Indicates no corresponding symptoms; Different lowercase letters in the same column indicate significant difference among the treatments at 0.05 level

**Table 6. Induction of explant buds by different hormone combinations.**

| Experimental treatments | Concentration of NAA (mg/L) | Concentration of 6-BA (mg/L) | Inoculation number | Budding number | Bud rate (%) |
|---|---|---|---|---|---|
| B0 | 0 | 0 | 60 | 10 | 16.7 ± 10.4d |
| B1 | 0.2 | 0.5 | 60 | 37 | 61.7 ± 10.4bc |
| B2 | 0.2 | 1 | 60 | 46 | 76.7 ± 2.9abc |
| B3 | 0.2 | 1.5 | 60 | 48 | 80.0 ± 18.0ab |
| B4 | 0.2 | 2 | 60 | 55 | 91.7 ± 14.4a |
| B5 | 0.5 | 2 | 60 | 58 | 96.7 ± 2.9a |
| B6 | 1.0 | 2 | 60 | 45 | 75.0 ± 10.0abc |
| B7 | 1.5 | 2 | 60 | 40 | 66.7 ± 16.1bc |
| B8 | 2 | 2 | 60 | 34 | 56.7 ± 7.6c |

Notes: Different lowercase letters in the same column indicate a significant difference among the treatments at 0.05 level

**Table 7. induction of naked capsule root by different hormone combinations.**

| Experimental treatments | Inoculation number | Number of plants take root | Rooting rate (%) | Average root number | Average root length (cm) |
|---|---|---|---|---|---|
| C1 | 15 | 0 | 0.0 ± 0.0d | 0.0 ± 0.0d | 0.0 ± 0.0d |
| C2 | 15 | 0 | 0.0 ± 0.0d | 0.0 ± 0.0d | 0.0 ± 0.0d |
| C3 | 15 | 3 | 20 ± 20.2c | 9.3 ± 1.5b | 1.6 ± 0.5c |
| C4 | 15 | 2 | 13.3 ± 0.0cd | 3.0 ± 2.6c | 0.4 ± 0.4d |
| C5 | 15 | 0 | 0.0 ± 0.0d | 0.0 ± 0.0d | 0.0 ± 0.0d |
| C6 | 15 | 5 | 33.3 ± 11.5b | 11.0 ± 2.0b | 2.5 ± 0.6b |
| C7 | 15 | 10 | 66.7 ± 13.3a | 19.3 ± 2.5a | 4.8 ± 0.7a |
| C8 | 15 | 0 | 0.0 ± 0.0d | 0.0 ± 0.0d | 0.0 ± 0.0d |

Notes: Different lowercase letters in the same column indicate a significant difference among the treatments at 0.05 level

**Table 8. optimization of rooting medium with different hormone combinations.**

| Experimental treatments | (mg/L) Concentration of NAA (mg/L) | (mg/L) Concentration of IBA (mg/L) | Inoculation number | Number of plants take root | Rooting rate (%) | Average root number | Average root length (cm) |
|---|---|---|---|---|---|---|---|
| D1 | 0.5 | 0.8 | 9 | 0 | 0.0 ± 0.0c | 0.0 ± 0.0d | 0.0 ± 0.0d |
| D2 | 0.5 | 1 | 9 | 3 | 33.3 ± 33.4bc | 13.0 ± 11.4bc | 2.0 ± 1.8c |
| D3 | 0.5 | 1.5 | 9 | 6 | 66.7 ± 0.0ab | 19.0 ± 3.6b | 1.4 ± 0.5cd |
| D4 | 1 | 0.8 | 9 | 5 | 55.6 ± 19.3ab | 24.0 ± 1.7ab | 4.2 ± 0.3b |
| D5 | 1 | 1 | 9 | 8 | 88.9 ± 19.2a | 30.7 ± 2.1a | 6.6 ± 0.6a |
| D6 | 1 | 1.5 | 9 | 2 | 22.2 ± 38.5bc | 5.0 ± 8.7cd | 0.3 ± 0.6d |

Notes: Different lowercase letters in the same column indicate a significant difference among the treatments at 0.05 level

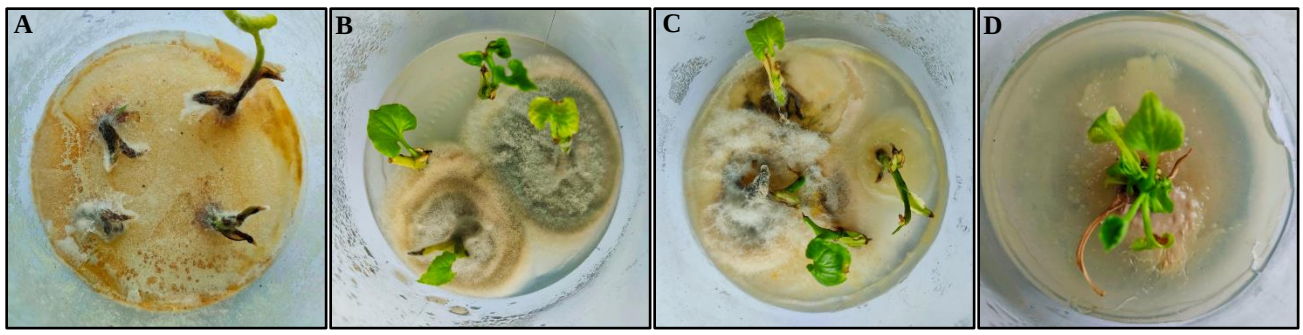


Fig. 1. The explants were contaminated by fungi and bacteria, after 3 weeks of culture: A & B, only fungal contamination; C: both fungal and bacterial contamination; D, only bacterial contamination.

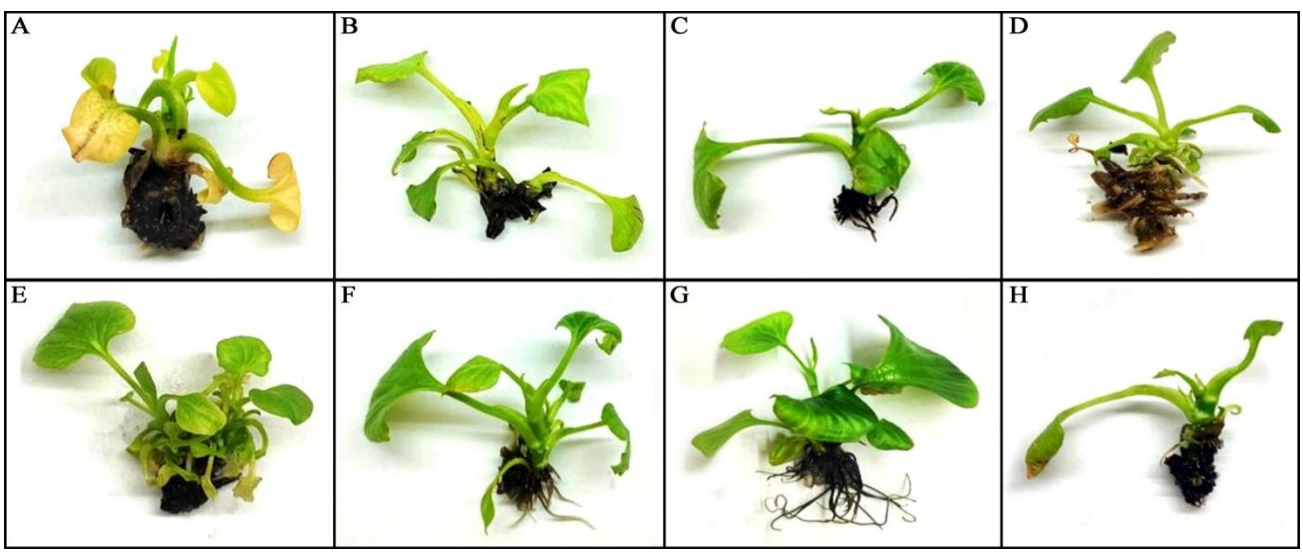


Fig. 2. Primary induction of rooting of naked capsule by different hormone combinations. (A~H: C1~C8, respectively).

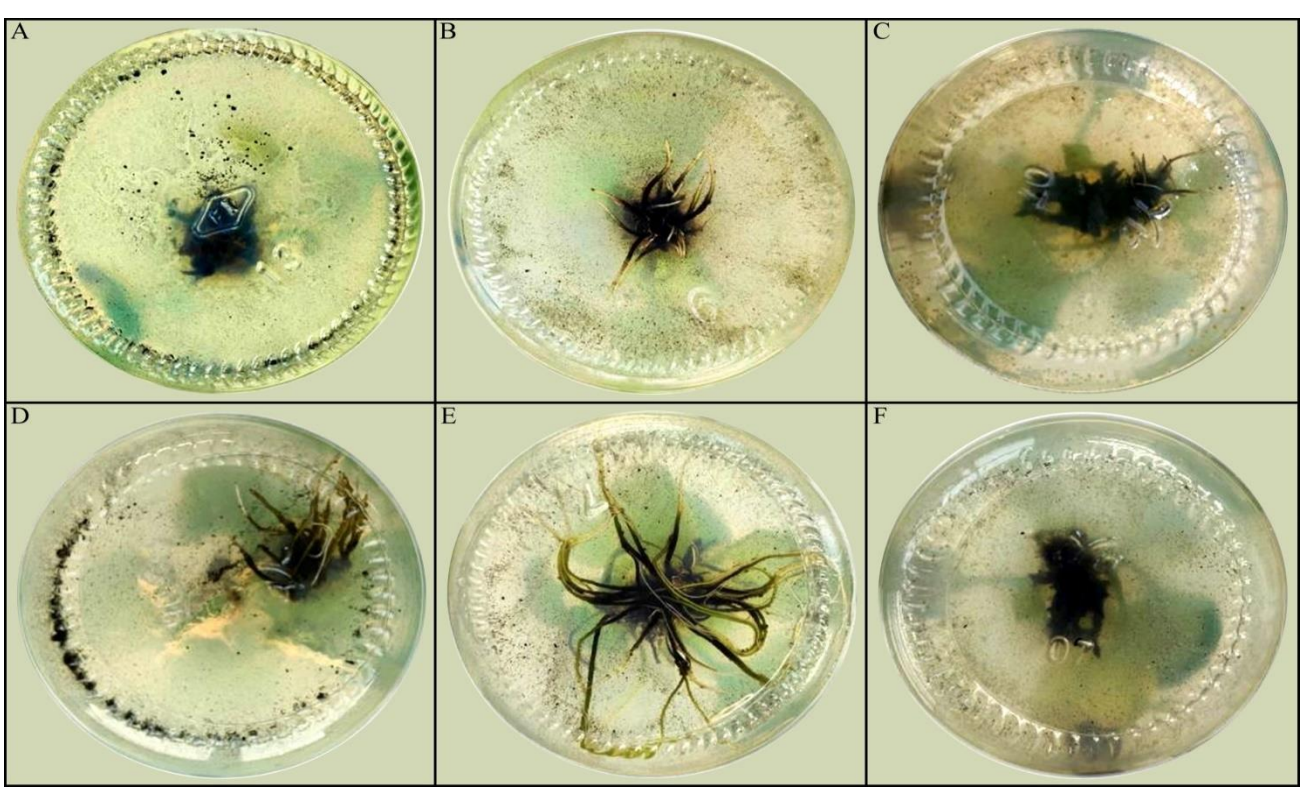


Fig. 3. Optimization of rooting medium with different hormone combinations (The black dots are activated carton in the picture: A~F: D1~D8, respectively).

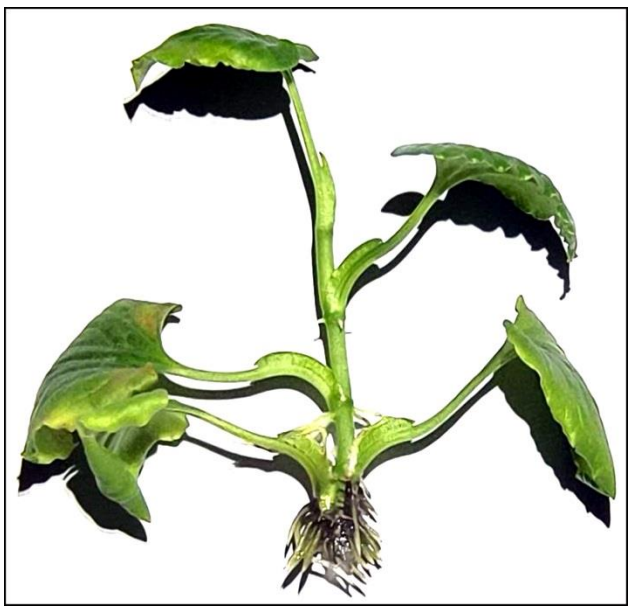

Fig. 4. *G. chinensis* seedlings in optimization combinations of medium.

**Screening of replacement medium:** According to the results of the first induction of bare capsule rooting medium, the best subculture medium was selected. The rooting of bare capsules was observed by regulating the concentration of IBA up and down. As can be seen from the results in Table 8, at a concentration of 1 mg/L for NAA and 1 mg/L for IBA concentration, the rooting rate reached 88.9% (treatment D5), the average number of roots increased to 30.7 roots, and the root length grew to 6.6 cm. When the concentration of NAA was controlled to be 0.5 mg/L, the rooting rate gradually increased with the gradual increase of the concentration of IBA; the rooting rate of D3 was increased to 66.7%; while the rooting rate of D1 was as low as 0, which was significantly different from that of D2 and D3. When the concentration of NAA was 1 mg/L, too much or too little concentration of IBA affected the rooting rate, and the concentration of IBA was increased to 1.5 mg/L, the rooting rate was only 22.2% (treatment D6), and the average number of roots and root length were significantly different from those of D4 and D5. Combined with the results in Fig. 3, it can be seen that in Fig. E under D5 treatment, the adventitious roots were robust and longer, and the plants were healthy. When the seedlings were transferred to the medium of D5 treatment, milky white root protrusions were visible as soon as the 15th d. After another week or so (Fig. 4), the number of adventitious roots and root length increased dramatically. Therefore, MS+1.0 mg/L NAA+1.0 mg/L IBA was the best medium for succession.

**Domestication and transplanting:** After 25 d of successive culturing, the growth of group-cultured seedlings basically reached the level of transplanting. After the domestication of *G. chinensis* group cultivated seedlings, the addition of humus as the substrate growth are better than the all-limed loess culture (Fig. 5), the survival rate also reached 100%, and its overall growth condition: E3>E2>E1. In treatment E1, the growth was slow and the leaves were yellowish in colour but appeared to be branched (Fig. 5E1); in treatment E3, it was able to adapt to the environment of the soil very quickly and grew rapidly, with robust, tall and stout plants and dark green leaves (Fig. 5E3). Therefore, the best results can be obtained by planting *G. chinensis* group culture in humus soil.

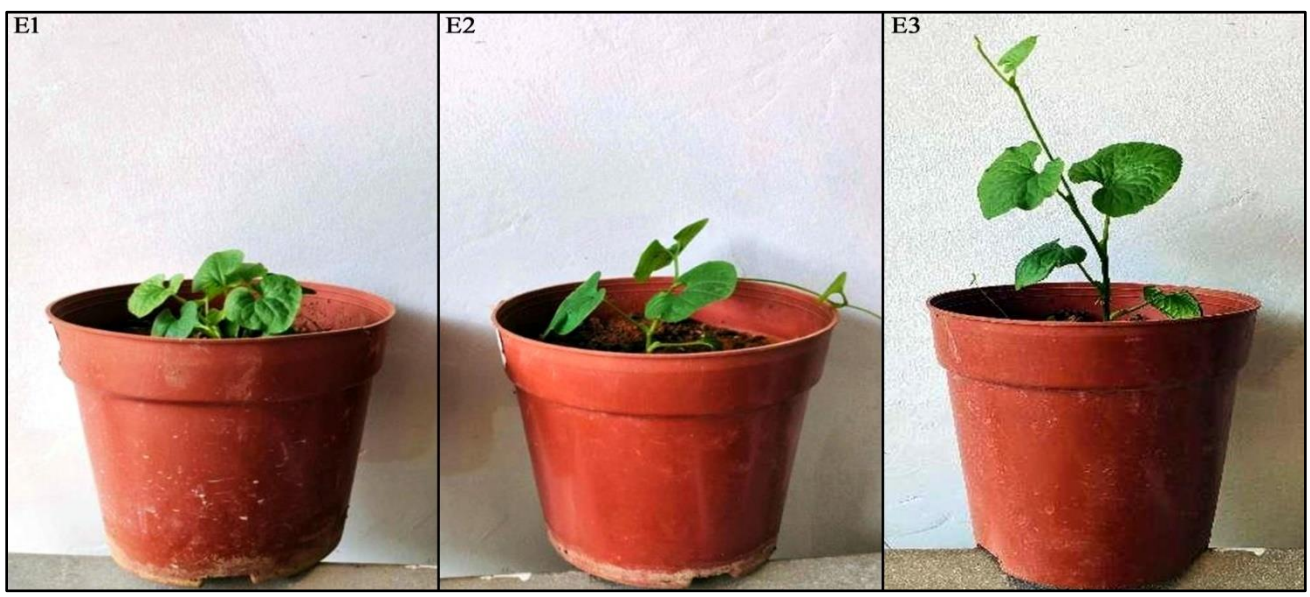


Fig. 5. Growth status of *G. chinensis* cordata at the 45 d. (E1: calcareous loess is matrix, E2: calcareous loess: humus =1:1 is substrate, E3: humus is substrate).

## Discussion

Plant tissue culture has the advantages of not being restricted by natural conditions and being able to accelerate the breeding process, which is of fundamental exploratory significance for the cultivation and promotion of medicinal plants. The regeneration system established in this study largely overcomes the limitations of endophyte enrichment cultivation difficulties and provides a new way for the scale breeding of naked capsules and the production and development of active ingredients. In the cultivation process, the direct induction of buds can greatly reduce the

cultivation period, minimise the mutation that occurs in the propagation process, and ensure the genetic stability of the plants between the mother lines.

The first step in the tissue culture system is to obtain sterile explants. The selection of appropriate disinfection methods and explants is of great significance in the study of plant tissue culture (Sun *et al.*, 2023). The combined use of several disinfectants performs excellently in the histoculture system of many plants (Bhadrawale *et al.*, 2018), Ying *et al.*, (2009) found that the use of 70% alcohol immersion for 30 s+0.1% $HgCl_2$ for 10-20 min + penicillin + streptomycin for 15 min sterilized the stems of *Cichorium erythrorhizonticum* much better than that of a single disinfectant , which is consistent with the results of this study. However, the contamination occurred almost a week later, mostly possibly caused by endophytes, and the control of endophytes in histoculture is done by using a antibiotics immersion (Ye *et al* ., 2022), two-step sterilization method with (Chen *et al.*, 2018)and addition of sterilizing agent to the culture medium (Behrendorff *et al.*, 2020), and so on. In this paper, stems and terminal buds were selected as explants, and the results of contamination and browning rates under the same disinfection method and the same culture environment showed that the terminal buds were much more successful as explants than stems. Finally, the nude capsule terminal bud was used as the explant, and it was soaked with 0.5 mg/L penicillin + streptomycin mixture solution for 3 h, treated with 75% ethanol for 30 s, and co-treated with 0.1% $HgCl_2$, and the best effect was achieved by using 0.1% $HgCl_2$ for two-step sterilization. It can be seen that for different plants as well as different explants, even if the same disinfectant is used, the disinfection time will be different, probably because different plants and explants have different tolerance to disinfectants, and prolonged exposure of more sensitive explants to disinfectants will result in damage to the explants. With the inactivation of disinfection for a longer time, the degree of browning will become serious, browning due to the OPP plays a key role (Coseteng & Lee, 2010), will be added to the medium with a small amount of activated charcoal, because activated charcoal has a certain degree of adsorption, can adsorb polyphenol oxidase oxidised substances, can reduce the degree of browning.

Plant growth regulators play a key role in plant tissue culture (Yang *et al.*, 2024), which can not only effectively regulate the physiological process of plants but also enhance plant resistance and improve plant quality and different doses of plant growth regulators have different impacts on plant differentiation, division, and development (Mou *et al.*, 2017). The reasonable ratio of exogenous growth regulators is the key to induce healing tissues and proliferate adventitious shoots, and Han *et al*., (2023) showed that MS+2.0 mg/L 6-BA+0.1 mg/L NAA was suitable for the growth of *Gentiana rhodantha* shoots. In this study, different concentrations of 6-BA and NAA were used in combination, and the top buds were inoculated on the medium of MS+6-BA 2 mg/L+NAA 0.5 mg/L, which could induce adventitious buds well. The bud rate reached 96.7 %, and the adventitious buds were directly induced from the top buds of the buds, eliminating the step of dedifferentiation to form callus. Compared with the use of leaves, inflorescences, and branches to induce callus first and then induce adventitious buds, the time of seedling raising was shortened, and the process of obtaining tissue culture seedlings was accelerated. The results are similar to those of Mei *et al.*, (2012) who used buds as explants. The results of the study showed that too large or too small ratio of hormones can affect the induction of adventitious shoots, so a reasonable ratio is the key.

Rooting directly determines the final part of the success of the histoculture fast multiplication system, and inducing healthy and strong roots can improve the adaptability of the seedlings to the outside world (Yuan *et al.*, 2025). The rooting ability of histocultured seedlings is affected by plant materials, basic medium, culture conditions and plant growth regulators, among which plant growth regulators play a decisive role in the rooting ability of histocultured seedlings (Xiao *et al.*, 2018). Some studies have been conducted to induce rooting using NAA, IAA+NAA and IBA+NAA, respectively, (Wang *et al.*, 2022). Therefore, in the present article, the roots of *G. chinensis* were induced using a combination of IAA, IBA and NAA. The rooting rate, average number of roots and average root length of NAA+IBA combination at suitable concentration were better than that of IAA+NAA Coseteng combination. Inoculation in MS+1.0 mg/L NAA+1.0 mg/L IBA medium resulted in a rooting rate of 88.9%, an average number of 30.1 roots, and an average root length of 6.6 cm. It shows that rational hormone combination can improve the root quality and improve the survival rate of cultivation, and provide ideas for tissue culture induced rooting in the future. When the primary medium was transferred into the rooting medium, it was found that the end of the inserted medium turned brown, because the trauma generated during the cutting of buds activated the polyphenol oxidase in the tissue. The enzyme was easily oxidized to brown quinones, which made the incision of the explants brown (Lee *et al.*, 2010). It would penetrate into the medium for a long time, causing the medium to brown, and seriously affecting the growth and differentiation of the culture. It would even lead to the death of the culture (Zhang *et al.*, 2020), timely transfer to new medium or excision contributes to the growth of bacterial seedlings.

## Conclusion

Tissue culture seedlings can improve the efficiency and quality of plant reproduction. In this paper, the top bud of naked capsule was selected as the explants and the mixture of 0.5 mg/L penicillin sodium + streptomycin ethanol for 30 s +20 min+0.1% $HgCl_2$ for 15 min, and MS+1.0 mg/L NAA+1.0 mg/L IBA+0.2 g/L activated carbon as the best secondary medium, successfully established *G. chinensis* tissue culture regeneration system. It well alleviates the problem of disease of naked capsule and is not affected by seasonality and provides basic reference for the subsequent large-scale seedling raising genetic transformation system and genetic engineering of *G. chinensis*.

## Acknowledgments

This work was supported by the Science and Technology Plan Project of Guizhou Province, China (QKHJC-ZK [2024]-YB426), the Key Field Projects of Guizhou Provincial Department of Education (QJHKY[2021]044), the Project of Guizhou Provincial Characteristic Key Laboratory (QJHKY[2021]002).

**References**


Aghaali, Z., Y. Hoshino, S.R. Monfared and A. Moieni. 2019. Regulation of dedifferentiation and differentiation in different explants of *Papaver rhoeas* L. by one-step culture. *Sci. Hort.*, 246(2019): 366-370.

Ahmadpoor, F., N. Zare, R. Asghari and P. Sheikhzadeh. 2022. Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in *In vitro* cultures *Melia azedarach* L. *AMB Express.*, 12(1): 3.

Akila, W.Y. and H. B. Jayeni. 2023. Tissue culture-A sustainable approach to explore plant stresses. *Life.*, 13(3): 780.

Anushi, S.J., M. Rathod, G. Mishra, V.L.P. Kumari, H. Baksh, S. Saxena and L. Prasad. 2023. Plant tissue culture for medical therapy: unlocking the potential of medicinal plants. *Curr. J. Appl. Sci. Technol*., 42(46): 7-22.

Behrendorff, J.B., G. Borràs-Gas and M. Pribil. 2020. Antimicrobial solid media for screening non-sterile *Arabidopsis thaliana* seeds. *Physiol. plant*., 169(4): 586-599.

Bhadane, B.S. and R.H. Patil. 2016. Data on the cost-effective surface sterilization method for *Carissa carandas* L. seeds and callus induction from aseptic seedling. *Data Brief.*, 7(C): 1551-1555.

Bhadrawale, D., J.P. Mishra and Y. Mishra. 2018. An improvised *In vitro* vegetative propagation technique for *Bambusa tulda*: influence of season, sterilization and hormones. *J. For. Res.*, 29(4): 1069-1074.

Chen, G., D. Chen, Q. Wang, Q. Hu, C. Huang, F. Huang and X. Ye. 2018. Tissue culture and rapid propagation of shoot-producing bamboo and seeding industriulization. *Agric. Eng*., 8(11): 1994-2023.

Chen, H.J., J.N. Liu, X. Li, S.J. Chen and C. Gang. 2018. Research progress on chemical constituents and biological activities of *Gymnotheca chinensis* Decne. *Chin*. *Trad. Pat. Med*., 40(5): 1150-1155.

Coseteng, M.Y. and C.Y. Lee. 2010. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci.*, 52(4): 985-989.

Danova, K. and L. Pistelli. 2022. Plant tissue culture and secondary metabolites production. *Plant.*, 11(23): 3312.

Deng, Y.W., J.Y. Lin, Q.N. Wu, T.T. Liu, M.Q. Liu and X.M. Deng. 2018. Overview in Tissue Culture of Magnolia. *For. Env. Sci*., 34(5): 7.

Golovatskaya, I.F., Y.V. Medvedeva, M.K. Kadyrbaev and E.V. Boyko. 2024. Specificity of growth and accumulation of flavonoids in plants and cell cultures of *Lychnis chalcedonica* obtained from explants of different organs. *Russ. J. Plant Physiol*., 71(1): 24.

Han, S., M. Liu, Y. Wang and J. Chen. 2023. Tissue culture and rapid propagation technology for *Gentiana rhodantha*. *Open Life Sci*., 18(1): 20220565.

Hassaan, M A. and A.E. Nemr .2020. Pesticides pollution: Classifications, human health impact, extraction and treatment techniques. *Egypt. J. Aquat. Res.*, 46(3): 207-220.

Jin, L.H., D.Y. Yang, X. Pei, X. Wei and M. Ni. 2015. Antibacterial activities against rice bacterial leaf blight and tomato bacterial wilt of 2-mercapto-5-substituted-1,3,4-oxadiazole/thiadiazole derivatives. *Bioorg. Med. Chen. Lett*., 25(3): 481-484.

Kandel, S.L., P.M. Joubert and S.L. Doty. 2017. Bacterial endophyte colonization and distribution within plants. *Microorgan.*, 5(4): 77.

Lee, M.R., J.K. Tweed, A. Cookson and M.L. Sullivan. 2010. Immunogold labelling to localize polyphenol oxidase (PPO) during wilting of red clover leaf tissue and the effect of removing cellular matrices on PPO protection of glycerol-based lipid in the rumen. *J. Sci. Food Agric*., 90(3): 503-510.

Lin, Q., Y. Wang, M.M. Li, Z.X. Xu and L. Li. 2022. Ecological niche selection shapes the assembly and diversity of microbial communities in *Casuarina equisetifolia* L. *Front. Plant Sci*., 13: 988483.

Liu, M., M. Bai, J. Yue, X. Fei and X. Xia. 2023. Integrating transcriptome and metabolome to explore the growth-promoting mechanisms of GABA in blueberry plantlets. *Front. Plant Sci*., 14: 1319700.

Mei, W., L. Ming, N. Xiaopei, L. Xingming and C. Li. 2012. Technical regulations for tissue culture of phalaenopsis. *Agric. Eng. Technol*., (02): 44-45.

Mitter, E.K., R. Kataoka, J.R. de Freitas and J.J. Germida. 2019. Potential use of endophytic root bacteria and host plants to degrade hydrocarbons. *Int. J. Phytorem.*, 21(9): 928-938.

Mou, H.F., J.Y. Liu, Y.C. Hhang, F.B. Deng, Y.Y. Wang, W.H. Hu, D.D. Wei, G.D. Li and L.F. Hu. 2017. Study on stem disinfection, bud induction and proliferation of *Hylocereus undatus In vitro* cultur. *S.W. Chin. J. Agric. Sci*., 30(06): 1439-1444.

Perelló, A., D. Olmo, A. Busquets, A. Romero-Munar, B.M. Quetglas, P.A. Gost, M. Berbegal, J. Armengol, C. Cabot and M. Gomila. 2024. First report of shoot blight of grapevine caused by *Sclerotinia sclerotiorum* in Illes Balears, Mallorca, Spain. *Plant Dis*., 108(4): 1101.

Sun, W., D. Yang, Z.M. Chen, Y. Zhu, Z.F. Wang, J. Li and D. Zhao. 2023. Tissue culture of the semi-mangrove plant *Hibiscus sabdariffa*. *J. Taizhou Univ.*, 45(03): 30-35.

Tam, H.T., H.T.M. Ngan, N.T.K. Cuc and D.L. Thanh. 2021. Pilot-scale culture of adventitious root for the production of pharmacology active from medicinal plants: a mini review. BIO Web of Conferences, 40: 03003.

Titov, S., S.K. Bhowmik, M.S. Alam and S.N. Uddin. 2007. Control of endogenous bacterial contamination and micropropagation of a traditional table banana (*Musa* spp·cv·Kanthali) of Bangladesh. *Chin. J. Biotechnol*., 23(06): 1042-1048.

Tung, H.T., T.T. Thuong, D.M. Cuong, V.Q. Luan, V.T. Hien, T. Hieu, N.B. Nam, H.T.N. Phuong, V.T.V. Bui and H.D. Khai. 2021. Silver nanoparticles improved explant disinfection, *In vitro* growth, runner formation and limited ethylene accumulation during micropropagation of strawberry (*Fragaria×ananassa*). *Plant Cell Tiss. Org*., 145(2): 393-403.

Wang, J.-X., T.T. Xu, J.S. Ji and X.C. Zao. 2019. Research progress in tissue culture of *Artemisia annua* L. *Mod. Agric. Sci. Technol*., (17): 69-72.

Wang, Y., Z.N. Yang, S.Q. Luo, Q. Ding, S. Yang and K. Wang. 2022. Establishment of tissue culture regeneration system of *Artemisia annua* L. *Mol. Plant Breed*., 1-17.

Wen, T.C., Z.Y. Qian and B.Y. Li. 2024. Research progress on explant sterilization technology of plant tissue culture. *Mod. Agric. Sci. Technol*., (13): 72-76

Xiao, H.J., J.L. Pang, L.Q. Meng, J. Zhang and P. Li. 2018. Screening of the culture medium for blueberry rooting in bottles. *Mol. Plant Breed*., 16(08): 2583-2586.

Xiao, S.J., D.L. Guo, M.S. Zhang, F. Chen, L.S. Ding and Y. Zhou. 2016. Chemical constituents of *Gymnotheca chinensis. Chin. Trad. Herb. Drug.*, 47(10): 1665-1669.

Xiao, S.J., D.L. Guo, M.S. Zhang, F. Chen, L.S. Ding and Y. Zhou. 2016. Three new oxazoline alkaloids from *Gymnotheca chinensis*. *J. Asian Nat. Prod. Res.*, 18(8): 719-723.

Yang, A., Y. Chen, S. Zhu, L. Jiang, J. Chen, C. Li, P. Li, W. Zeng, D. Kuang and Q. Liu. 2024. The impacts of plant growth regulators on the rapid propagation of *Gardenia jasminoides* Ellis. in tissue culture. *Forest.*, 15(3): 446.

Ye, H.T., S.Q. Luo, Z.N. Yang, Y.S. Wang and Q. Ding. 2022. Establishment of rapid propagation system on *Houttuynia cordata* Thunb. *Plant Breed*., 20(03): 951-958.

Ying, M., Y. Yuan and B. He. 2009. Sterilization of *Houttuynia cordata* Thunbn explant in tissue culture. *Anhui Agric. Sci. Bull*., 15(04): 27-29.

Yuan J.Y., T.Y. Zhang, Y.F. Hu, L. Bai, L. Zhang, J.T. Yang, Y.H. Zhang, Y.A. Qin, Z.H. Xu and T. Han. 2025. Effects of plant growth regulators and endogenous phytohormone profiles on the rooting of tissue culture raised shoots of persimmon (Diospyros kaki Thunb.). *Sci. Hort*., 339(2025): 113917.

Zhao,P. Y., Y.N. Tang, S.W. Liu, X.R. Wang, F.J. Xu, L.Y. Ruan, J.L. You and X.Y. Meng. 2022. Herbal Textual Research of *Saururus Chinensis* Lour Baill. *APAC. Trad. Med*., 18(7): 175-183

Zhang, F., L. Liu, J.R. Huan and M.l. Cai. 2020. *In vitro* propagation of *Artemisia annua* L. *J. Green Sci. Technol*., (01): 11-13.

Zhang, P.Z, M.S. Su, Y.Q. Wang, F. Shao, M. Yang and Y.M. Zhang. 2016. Chemical constituents from *Gymnotheca involucrata*. *Chin. Trad. Herb. Drug*., 47(19): 3366-3369 .

Zhang, Q.F., Q.J. Li, M.J. Ma, D.Q. Xiao, J. Zhao and J.K. Yang. 2022. Common problems and countermeasures in plant tissue culture. *Contemp. Hort.*, 45(10): 177-179.

(Received for publication 4 June 2024)