

Establishment of a Rapid Propagation System for *Waltheria indica* L. and Acquisition of Nematode-Repellent Active Compounds

Meihua Tang

Hainan University <https://orcid.org/0009-0002-6717-8113>

Xinyi Lu

Hainan University

Yongdie Gan

Hainan University

Sher Ahmed

Hainan University

Zhan Hu

Hainan University

Dong Li

Hainan University

Shuai Wang

996018@hainanu.edu.cn

Hainan University <https://orcid.org/0000-0002-6615-2491>

Ranfeng Sun

Hainan University

Research Article

Keywords: *Waltheria indica* L, Rapid Propagation System, Nematode-Repellent, Antidesmone, Trans-tiliroside

Posted Date: November 21st, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8033700/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

1 Establishment of a Rapid Propagation System for *Waltheria indica* L.

2 and Acquisition of Nematode-Repellent Active Compounds

3 Meihua Tang^{1#}, Xinyi Lu^{1#}, Yongdie Gan¹, Sher Ahmed¹, Zhan Hu¹,

4 Dong Li¹, Shuai Wang^{1*}, Ranfeng Sun^{1*}

5 1 Key Laboratory of Green Prevention and Control of Tropical Plant Diseases and Pests,
6 Ministry of Education, School of Tropical Agriculture and Forestry, Hainan University,
7 Danzhou, 571737, People's Republic of China

8 **Abstract:** This study aimed to establish an efficient tissue culture and rapid propagation
9 system for *Waltheria indica* L., to evaluate the potential of its tissue-cultured materials
10 producing active compounds with repellent activity against *Meloidogyne incognita*.
11 The proliferation of sterile seedlings was investigated using seeds as explants.
12 Adventitious buds and root induction from hypocotyls and leaves, adventitious root
13 suspension culture system was also established. The results showed that the optimal
14 sterilization method was 75% ethanol for 30 s followed by 15% NaClO for 15 min,
15 achieving non-contamination and 49.17% germination rate. The best medium for
16 seedling proliferation was MS + 0.05 mg/L 6-BA with a proliferation coefficient of 9.17,
17 while the optimal rooting medium was MS + 0.1 mg/L IAA, yielding a rooting rate of
18 96.67%. Adventitious buds and seedlings were induced from hypocotyls on MS + 0.1
19 mg/L 6-BA. Callus induction from leaves reached 100%, and adventitious roots were
20 induced from callus on MS + 1.0 mg/L 6-BA + 0.2 mg/L NAA + 1.0 mg/L 2,4-D. The
21 optimal suspension culture medium for adventitious roots was MS + 30 g/L sucrose +
22 1.0 mg/L NAA, with a relative proliferation multiple of 5.03, and the best subculture
23 time was 30 days after inoculation. Among the 12 compounds isolated from *W. indica*,
24 antidesmone and trans-tiliroside exhibited repellent effects on second-stage juveniles
25 of *M. incognita*, with chemotaxis index of 0.41 and 0.22, respectively at 10 mg/L.
26 Analysis of compound content revealed that suspension-cultured adventitious roots
27 significantly enhanced the relative content of antidesmone. Although tissue culture
28 conditions suppressed trans-tiliroside synthesis in stems and leaves, exogenous
29 application of 0.05 mg/L 6-BA effectively reversed this inhibition and promoted its
30 accumulation. This study establishes a rapid propagation system for *W. indica*,
31 providing a theoretical foundation for its large-scale production, accelerates breeding,
32 and enhances its secondary metabolite content.

Key message: This study established a rapid propagation system for *Waltheria indica* and simultaneously enhanced the content of compounds with nematode-repellent activity.

Keywords: *Waltheria indica* L; Rapid Propagation System; Nematode-Repellent; Antidesmone; Trans-tiliroside

Submission date: 2025-10-30

Fund project: Hainan Provincial Natural Science Foundation of China 325MS015 and 324QN207; Hainan University research fund project RZ2200001238; Key Laboratory of Plant Disease and Pest control of Hainan Province open project KF2022HN03.

*Correspondence authors: E-mail: 996018@hainanu.edu.cn; E-mail: srf18@hainanu.edu.cn.

Abbreviations

MS Murashige and Skoog Medium

PGR Plant Growth Regulator

IAA Indole-3-acetic acid

6-BA 6-Benzylaminopurine

IBA Indole-3-butyric acid

NAA 1-Naphthaleneacetic acid

2,4-D 2,4-Dichlorophenoxyacetic acid

HPLC High-Performance Liquid Chromatography

1. Introduction

Plant parasitic nematode disease is one of the important diseases that cause reduced crop yields. The losses caused by nematode damage exceed 170 billions US dollars every year. Root-knot nematode (*Meloidogyne* spp.) is one of the most important nematode genera. It has a wide host range and poses a serious threat to agricultural production worldwide (Khan et al, 2023; Azlay et al, 2023; Kavitha et al, 2025). The second-instar larvae (J2s) of root-knot nematodes can utilize their chemotaxis in the soil. By recognizing the chemicals released by plant roots or rhizosphere microorganisms, they can locate the host and migrate towards it, eventually successfully infecting the host (La et al, 2024; Tsai et al, 2021). Many plants can secrete some compounds to repel root-knot nematodes or interfere with signal substances to deplete the energy of the nematodes before they infect the host, thus preventing them from infecting the host plants and achieving the purpose of controlling nematodes (Beeman et al. 2016; Swaruparani et al. 2024; Terra et al. 2023). Modern research has found that common plants that can repel root-knot nematodes are mostly distributed in the Compositae, Brassicaceae, Liliaceae, and Apocynaceae families, etc. Natural phytochemicals that have been found to antagonize root-knot nematodes include

polythiophene, isothiocyanates, glucosinolates, cyanosides, alkaloids, terpenoids, flavonoids, steroids, phenols and several others (Kihika et al, 2017; Kirwa, et al, 2018; Ferreira, et al, 2025). Two alkaloids secreted by tomato seeds, dihydrocapsaicin and solanine can disrupt the localization of the host by *M.incognita* (Kuang et al, 2020). Interplanting of *Ricinus communis* can reduce the infection of *M. incognita* on tomato roots. Through gas chromatography-mass spectrometry (GC-MS) detection combined with in vitro chemotaxis tests, it was found that palmitic acid and linoleic acid in *R. communis* have repellent effects on the J2s of *M. incognita*. Moreover, as the compound concentration increased (0.5-4.0 mmol/L), the repellent effect on J2s gradually strengthened, and at the same time, the expressions of the key olfactory regulatory genes *Mi-flp-18* and *Mi-mpk-1* were down-regulated (Dong et al, 2018). It was found that the roots of *Waltheria indica* have a very strong repellent effect on the J2s of *M. incognita* in the Pluronic F-127 gel. The waltherione A and waltherione C in the *W. indica* are highly toxic to the root-knot nematodes (Liang et al, 2019; Jang et al, 2019). However, no study has reported the active repellent substance in *W.indica* and its concentration relationship against root-knot nematodes.

W. indica L. Sterculiaceae belongs to the *Waltheria* genus of the Sterculiaceae family. It is a slightly upright or prostrate semi-shrub plant. This genus contains more than 60 species worldwide, which are widely distributed in tropical and subtropical regions such as Africa, South America and Southeast Asia (Zong et al,2013; Yabré et al,2024). There is only one specie in China, namely *W. indica*, which is mainly distributed in Fujian, Taiwan, Guangdong, Hainan, Guangxi, Yunnan provinces and other places (Liang et al,2019). Research has found that the *W. indica*. contains over 60 compounds such as flavonoids, alkaloids and terpenoids. The *W. indica* has anti-inflammatory, antibacterial, anti-trypanosomiasis and anti-cancer effects (Gelfand L et al,1985; Nirmala C et al,2021; Zhou Z et al,2025). In addition, the secondary metabolite waltherione A has control effect on root-knot nematode activity (Liang C et al,2019), the antidesmone has antifungal activity (Jang J Y et al,2019; Yin F et al,2021). Therefore, it is of great significance to establish a rapid propagation system for *W. indica* and form a technical process for quickly obtaining active compounds from *W. indica*. However, there are no relevant research reports on tissue culture technology of *W. indica* and the large-scale production of active compounds using its tissue culture technology.

Plant tissue culture technology refers to the process of culturing isolated plant

organs, tissues, cells, embryos, and protoplasts on artificially prepared culture media under sterile conditions, providing suitable culture conditions to induce the formation of callus, latent buds, or complete plants (Sarma M K et al,2023). Plant tissue culture technology has multiple uses. On the one hand, it can study the mechanisms of plant growth, development and differentiation for genetic transformation, which is of special significance for plant genetic breeding (Su W et al; 2023). On the other hand, it can be used for large-scale factory production of useful compounds, for cell culture screening of useful clones in industry, agriculture, and pharmaceutical production, or for the source of protoplasts in protoplast culture, etc (Gulzar et al; 2020; Ravindra BM et al,2025). Plant tissue culture is applied to the *in vitro* rapid propagation of plants and is currently the most widely used, extensive and effective technique (Manoj Kumar Tripathi et al; 2021). It is not affected by region or climate and can be tens of thousands to millions of times faster than conventional breeding methods (Ahmar S et al,2020). The advantages of rapid propagation methods lie in their low material usage, pure varieties, no bacterial carrier, fast growth, high yield, and high commercial value (Babich et al; 2020). They can produce a large number of high-quality seedlings in a short time and within a very limited space to supply the market, achieving significant economic benefits (Sehgal D et al,2020). Practice has proved that plant tissue culture is not only a new means of rapid plant propagation, but also an ideal approach for plant improvement, germplasm preservation and the production of active compounds (Khezri et al, 2024).

The traditional reproduction method of *W. indica* is mainly seed reproduction, which effectively preserves its excellent traits. However, it also has problems such as being restricted by seasons, low seed germination rate, slow growth rate, and significant differences in the content of medicinal components among individuals, which are not conducive to large-scale production. During the normal growth and development of *W. indica*, the production amounts of active secondary metabolites (Waltherione A, Antidesmone and Trans-tiliroside) are difficult to meet the increasing medicinal demands. Therefore, establishing an efficient tissue culture and rapid reproduction system for the *W. indica* can effectively solve the problem of the shortage of natural resources for the *W. indica*. At present, the research on *W. indica* mainly focuses on the extraction of chemical components and its biological activities. There are few reports on the plant tissue culture of *W. indica*. Establishing a plant tissue culture system of *W. indica* is of great significance for accelerating the breeding of *W. indica* and increasing

the content of secondary metabolites in it.

2. Materials and Methods

2.1 Source of materials

Seeds of *W. indica* were collected from the New Agricultural Science Practice Base of Hainan University in Danzhou, Hainan Province. Healthy mother plants were selected, and the seeds were manually cleaned to remove debris (e.g., leaves and stones). Then the seeds were stored in a dry, cool, and well-ventilated environment. Compounds of *W. indica* were isolated and purified from the ethanol extract of the *W. indica*. All plant hormones, biological reagents were purchased from Sangon Biotech Co., Ltd. All organic solvents used in the study were of analytical grade and purchased from Aladdin or Tokyo Chemical Industry Co.

2.2 Subculture and proliferation of *W. indica* sterile seedlings

Seeds of the *W. indica* were chosen as explants and the process of sterilization of the exogenous organisms is as follows: Seeds were selected based on full maturity and plumpness, rinsed 5 times with sterile water (5 min per rinse), and air-dried naturally. Subsequently, they were transferred to a sterile workbench for disinfection using 75% ethanol and NaClO solution. Four treatment groups with varying disinfectant parameters were established (Table 1), and after disinfection, the seeds were rinsed with sterile water 10 times (2 min per rinse). Surface moisture was removed with sterile filter paper, sterilized seeds were placed evenly on MS solid medium using sterile forceps. Each treatment was replicated in 10 culture vessels, with 20 seeds inoculated per vessel. Culture conditions: temperature 26-28°C, photoperiod L/D=12/12 h, light intensity 1500-2000 lux, relative humidity 70%. Data were collected after 20 days of culture. Contamination rate and germination rate of *W. indica* were calculated according to the following formula.

$$\text{Contamination rate (\%)} = \frac{\text{Number of contaminated explants}}{\text{Number of inoculated explants}} \times 100\%$$

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of inoculated seeds}} \times 100\%$$

After 30 days of culture, sterile seedlings exhibiting uniform growth were selected. The seedlings were cut into 3 cm stem segments with 2 axillary buds and inoculated onto MS medium supplemented with varying PGRs for proliferation culture. Seventeen treatment groups with different PGR combinations were established (Table 2). Each treatment was replicated in 15 culture vessels, with 2 stem segments inoculated per

vessel. Culture conditions: temperature 26-28°C, photoperiod L/D=12/12 h, light intensity 1500-2000 lux, relative humidity 70%. Data on proliferation coefficients were collected after 60 days of culture.

$$\text{Proliferation coefficient} = \frac{\text{Total number of buds after proliferation}}{\text{Total number of buds at inoculation}}$$

Table 1 Disinfection Methods of Different Treatment Groups

Serial Number	Ethanol (%)	Disinfection time(s)	NaClO (%)	Disinfection time(min)
1	75	30	15	10
2	75	30	15	13
3	75	30	15	15
4	75	30	20	10

Table 2 Different Proliferation Treatment Groups of PGRs Ratio

Serial Number	IAA(mg/L)	6-BA(mg/L)	Number of vaccinations
A	0	0	30
B	0	0.05	30
C	0.02	0.05	30
D	0.05	0.05	30
E	0	0.1	30
F	0.02	0.1	30
G	0.05	0.1	30
H	0.02	0	30
I	0.05	0	30
J	0	0.5	30
K	0.1	0.5	30
L	0.2	0.5	30
M	0	1.0	30
N	0.1	1.0	30
O	0.2	1.0	30
P	0.1	0	30
Q	0.2	0	30

2.3 Root cultivation of the *W. indica* branch

Sterile seedlings with optimal growth after propagation were selected and divided into single branch, then the branches were inoculated into MS medium supplemented with varying PGRs for rooting culture. Six treatment groups with different PGR combinations were established (Table 3). Each treatment was replicated in 15 culture vessels, with 2 branches inoculated per vessel. Culture conditions: temperature 26-28°C, photoperiod L/D=12/12 h, light intensity 1500-2000 lux, relative humidity 70%. Rooting rates were collected after 30 days of culture as the following formula.

$$\text{Rooting rate (\%)} = \frac{\text{Number of rooting explants}}{\text{Total number of inoculated explants}} \times 100\%$$

Table 3 Ratio of PGRs in different rooting treatment groups

Serial Number	IBA(mg/L)	IAA(mg/L)	Number of vaccinations
1	0	0	30
2	0.5	0	30
3	1	0	30
4	1.5	0	30
5	0	0.1	30
6	0	0.2	30

2.4 Adventitious buds induction and seedling formation from hypocotyls of *W. indica*

The well-growing hypocotyls of *W. indica* were cut into 1cm segments and inoculated into an induction medium (MS basal medium supplemented with 0.1 mg/L 6-BA). Cultures were observed every 10 days. Culture conditions: temperature 26-28°C, photoperiod L/D=12/12 h, light intensity 1500-2000 lux, relative humidity 70%.

2.5 Induction of callus and adventitious roots from leaves of *W. indica* sterile seedlings

The leaves of sterile seedlings were excised and cut into 1×1 cm explants, then explants were inoculated onto callus induction medium (MS basal medium supplemented with 1.0 mg/L 6-BA, 0.2 mg/L NAA, and 1.0 mg/L 2,4-D). Observations were recorded at 5-day intervals. The induced callus were subsequently transferred to proliferation medium (MS basal medium containing 0.1 mg/L NAA) and similarly observed every 5 days. Culture conditions: temperature 26-28°C, photoperiod L/D=12/12 h, light intensity 1500-2000 lux, relative humidity 70%. Callus induction rate was calculated as the following formula.

$$\text{Callus induction rate (\%)} = \frac{\text{Number of explants forming into callus}}{\text{Total number of inoculated explants}} \times 100\%$$

The proliferating callus tissue was transferred and inoculated onto differentiation medium (MS basal medium supplemented with 1.0 mg/L 6-BA, 0.2 mg/L NAA, and 1.0 mg/L 2,4-D). Then adventitious root observations were recorded every 10 days, while culture conditions were maintained temperature 26-28°C, photoperiod L/D=12/12 h, light intensity 1500-2000 lux, relative humidity 70%.

2.6 Establishment of an adventitious root suspension culture system

The best PGR for adventitious root suspension were determined as following steps: Well-growing induced adventitious roots were excised and cut into 1 cm segments. Used MS as the basic culture medium, added sucrose 30 g/L, prepared the culture medium according to the concentration gradient of 0.1, 0.5 1.0, 2.0 mg/L NAA. (pH 5.8), 0.5 g root segments were inoculated into 50 mL medium in conical flasks. Each treatment included three replicate flasks. Flasks were maintained on a shaker (105 rpm) at 25 ± 2°C in darkness. Data were collected after 30 days.

The optimal inoculation amount of adventitious root suspension were determined as following steps: Well-growing adventitious roots were induced and cut into 1 cm segments. These root segments were then inoculated into 100 mL of liquid MS medium supplemented with 30 g/L sucrose and 1.0 mg/L NAA, at concentrations of 0.5%, 1%, 1.5%, 2%, 2.5%, and 3% (w/v). The cultures were incubated in the dark on a shaker at 105 rpm and a temperature of 25 ± 2°C. Data were collected after 30 days of cultivation. The net growth of adventitious root (g)=Fresh weight of adventitious root at harvest (g)-Initial fresh weight of adventitious root (g)

$$\text{Adventitious root relative proliferation multiple} = \frac{\text{Adventitious root net growth (g)}}{\text{Initial adventitious root fresh weight (g)}}$$

The growth curve of adventitious root suspension were determined as following steps: Well-growing induced adventitious roots were excised and cut into 1 cm segments. Precisely 0.5 g segments were inoculated into 50 mL liquid MS basal medium supplemented with 30 g/L sucrose and 1.0 mg/L NAA. Cultures were maintained on a shaker (105 rpm) at 25 ± 2°C in darkness. Adventitious root fresh weights were recorded at 5 days intervals over a 40 days culture period.

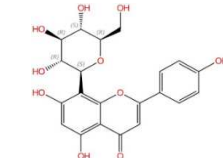
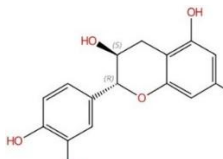
2.7 Detection of nematode chemotactic properties of compounds from *W. indica*

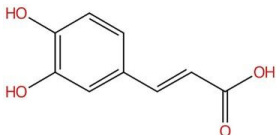
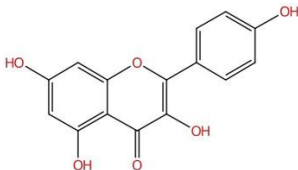
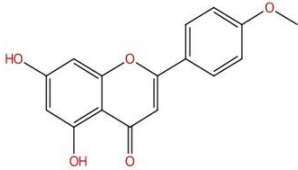
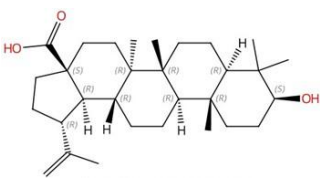
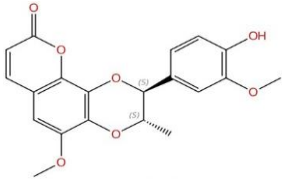
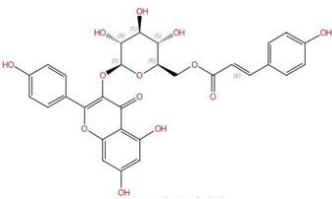
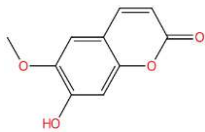
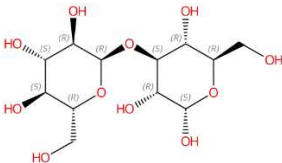
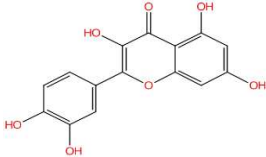
The test compounds were extracted from the *W. indica* (Table 4). The compounds were first dissolved in methanol. Then it was diluted to 23% of Pluronic solution.

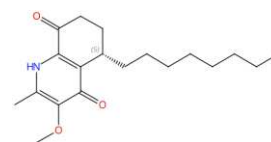
Referring to the method of Yuyang Wei (2023), a silica gel mold (40×20×5 mm) was placed in the bottom of a 60 mm diameter glass Petri dish, and a silica gel spacer was placed in the center of the mold to separate the area into two square areas of equal size. The concentration of 23% Pluronic gel solution with compound or with solvent were poured into the left and right sides of silica gel mold. After the Pluronic gel was solidified at room temperature, the silica gel spacer in the center was withdrawn, then 4 mm each of the left and right sides of the junction, a total of 8 mm width of Pluronic gels were scraped off. Then 0.5 mL of blank Pluronic gel was added into the blank area left as the buffer area. After complete solidification of the gel, 10 µL solution containing approximately 150 J2s of *M. incognita* was inoculated into the central buffer area of the gel. The silica gel molds were moisturized and placed in the dark at 25°C for 8 hours. After the *M. incognita* formed a trajectory in the gel, pictures were respectively taken on the planar surface of gel with compound and gel with solvent side at fixed magnification and parameters with a NIKON SMZ745T in-body microscope. Then pictures were processed by ImageJ, an image processing software, to capture images of the compound side of planar surface and the solvent side of the planar surface. The images were processed into black and white 8-bit images using the image processing software ImageJ, and the percentage of the nematode track in the number of pixels in the whole picture was calculated after processing and calculating the number of white pixels corresponding to the track, and the chemotaxis index (CI) was calculated according to the formula.

$$\text{Chemotaxis index} = \frac{2 \times \text{Treatment side pixel ratio}}{\text{Treatment side pixel ratio} + \text{Control side pixel ratio}}$$

Table 4 Compounds extracted from the *Waltheria indica* L.

Serial Number	Compound Name	CAS Number	Compound Structure
1	Vitexin	3681-93-4	
2	(+)-Catechin	154-23-4	

3	Caffeic acid	331-39-5	
4	Kaempferol	520-18-3	
5	Acacetin	480-44-4	
6	Betulinic acid	472-15-1	
7	(+)-Propacin	90523-83-4	
8	Trans-tiliroside	20316-62-5	
9	Scopoletin	92-61-5	
10	α -Nigerose	52485-08-2	
11	Quercetin	117-39-5	



2.8 Content detection of bioactive compounds in different tissues of *W. indica*

The collected roots, stem, and leaves of *W. indica* were dried and ground. For each part, 5 g of the ground material was extracted by adding dichloromethane and methanol. The extraction was repeated three times with immersion time of 24h, 48h, and 72h hours. Later extracts were combined, filtered, and concentrated to a crude paste using a rotary evaporator. The crude extract was stored at 4°C for subsequent analysis.

0.1 g crude extract of *W. indica* was dissolved in 10 mL HPLC-grade methanol. After vortex-mixing until complete dissolution, the solution was purified using C18 solid-phase extraction sorbent. The eluate was filtered through a 0.22 µm membrane and transferred to amber glass vials for analysis by an agilent UHPLC system. The chromatographic conditions were as follows: C18 column (4.6 × 250 mm, 5 µm, Welch Sci & Tech, Shanghai, China), column temperature: 30°C; flow rate: 1.0 mL /min; injection volume: 10 µL. For antidesmone analysis, UV detection wavelength: 254 nm; mobile phase: 80% methanol (HPLC-grade) and 20% ultrapure water. For trans-tiliroside analysis: UV detection wavelength: 313 nm, mobile phase: 55% methanol (HPLC-grade) and 45% ultrapure water.

2.9 Data Statistics

IBM SPSS and Microsoft Excel were used to analyze the experimental data.

3. Results and analysis

3.1 Subculture and proliferation results of *W. indica* sterile seedlings

Effects of different disinfection methods on explants as presented in table 5, NaClO concentration and exposure duration significantly affected explant contamination and germination rates. Disinfection with 15% NaClO for 15 minutes and 20% NaClO for 10 minutes both resulted in a 0% contamination rate. However, the germination rate of 15% NaClO disinfection for 15 min was 49.5%, which was significantly higher than that of 20% NaClO disinfection for 10 min, which was 35.5%. Combining the contamination rate and germination rate, the best way to disinfect the seeds of *W. indica* as explants was 75% alcohol disinfection for 30s + 15% NaClO disinfection for 15min. As presented in table 6, that different PGR ratios significantly

affected the axillary bud proliferation of *W. indica* sterile seedlings. The results showed that a higher concentration of 6-BA inhibited axillary bud proliferation. As the concentration of 6-BA increased, the proliferation coefficient of axillary buds decreased initially and then increased. In contrast, a lower concentration of 6-BA (0.05 mg/L) promoted proliferation, as indicated by an increase in the number of axillary buds and lateral branches (Fig. 1). Different concentrations of IAA had small effect on the growth of the seedlings. With increasing IAA concentration, the axillary bud proliferation coefficient first increased and then decreased. The combination of IAA and 6-BA did not exhibit a significant promoting effect on axillary bud proliferation (Fig. 1, Table 6).

As presented in Figure 1, different PGR treatments significantly affected the 60-day growth of the sterile seedlings. A high concentration of 6-BA inhibited plant growth, characterized by short plants and yellowing leaves. In contrast, a low concentration of 6-BA promoted the formation of axillary buds and lateral branches (Fig. 1B), whereas 0.02 mg/L IAA exhibited inhibitory effects (Table 6. Serial Number 8). Generally, the combination of IAA and 6-BA had minimal effect on plant growth. Based on the axillary bud proliferation coefficient and overall plant growth status, the optimal proliferation medium for *W. indica* sterile seedlings was determined to be MS medium supplemented with 0.05 mg/L 6-BA.

Table 5 Effects of different disinfection methods on explants

Serial Number	Ethanol (%/s)	NaClO (%/min)	Contamination rate(%)	Germination rate(%)
1	75/30	15/10	80±5.77b	21.00±6.58c
2	75/30	15/13	100±0.00a	11.00±6.58d
3	75/30	15/15	0±0.00c	49.50±8.96a
4	75/30	20/10	0±0.00c	35.50±3.69b

Note : Different lowercase letters indicate significant differences ($P < 0.05$).

315 Table 6 Effect of different PGR ratios on the proliferation of axillary buds in
 316 sterile seedlings

Serial Number	IAA (mg/L)	6-BA (mg/L)	Number of axillary buds after proliferation	Proliferation coefficient
A	0	0	8.2 ± 1.40 gh	4.10 ± 0.69 gh
B	0	0.05	18.4 ± 2.17 a	9.20 ± 1.09 a
C	0.02	0.05	8.8 ± 2.35 fgh	4.40 ± 1.17 fgh
D	0.05	0.05	12 ± 3.82 bcdefg	6.00 ± 1.91 bcdefg
E	0	0.1	10 ± 2.11 defg	5.00 ± 1.05 defg
F	0.02	0.1	10.6 ± 3.31 bcdefg	5.30 ± 1.65 bcdefg
G	0.05	0.1	10.4 ± 1.71 cdefg	5.20 ± 0.86 cdefg
H	0.02	0	6.2 ± 2.78 h	3.10 ± 1.39 h
I	0.05	0	10.4 ± 3.30 cdefg	5.20 ± 1.65 cdefg
J	0	0.5	13 ± 5.01 bcde	6.5 ± 2.51 bcde
K	0.1	0.5	14.1 ± 4.95 bc	7.05 ± 2.48 bc
L	0.2	0.5	12.6 ± 3.86 bcdef	6.30 ± 1.93 bcdef
M	0	1.0	13.5 ± 4.47 bcd	6.75 ± 2.24 bcd
N	0.1	1.0	14.5 ± 5.19 b	7.25 ± 2.60 b
O	0.2	1.0	14.3 ± 5.01 bc	7.15 ± 2.50 bc
P	0.1	0	13.2 ± 5.94 bcde	6.60 ± 2.97 bcde
Q	0.2	0	9.4 ± 3.59 efgh	4.70 ± 1.79 efgh

317 Note : Different lowercase letters indicate significant differences ($P < 0.05$).



Fig. 1 Effects of different PGRs on the growth of *W. indica* sterile seedlings for 60 days

3.2 Effects of different PGRs on rooting of *W. indica* branch

As presented in Table 7, PGRs significantly affected rooting of *W. indica* branches. Compared to the control (without PGR), only the treatment with 0.1 mg/L IAA significantly increased the rooting rate, reaching 96.67%. While other treatments containing IBA or 0.2 mg/L IAA reduced the rooting rate. The rooting situations of *W. indica* treated by the different PGRs were showed, and the roots generated by adding IBA were thinner and had fewer roots, while the roots generated by adding IAA were thicker and with more roots than CK (Fig. 2). In conclusion, the best rooting medium for *W. indica* branches was MS basal medium supplemented with 0.1 mg/L IAA.

Table 7 Effects of different PGRs on rooting of *W. indica* branch

Serial Number	IBA (mg/L)	IAA (mg/L)	Number of vaccinations	Rooting rate (%)
1	0	0	30	83.33±5.77b
2	0.5	0	30	70.00±10.00bc
3	1	0	30	53.33±5.77d
4	1.5	0	30	63.33±5.77cd
5	0	0.1	30	96.67±5.77a
6	0	0.2	30	80.00±10.00b

Note : Different lowercase letters indicate significant differences ($P < 0.05$).

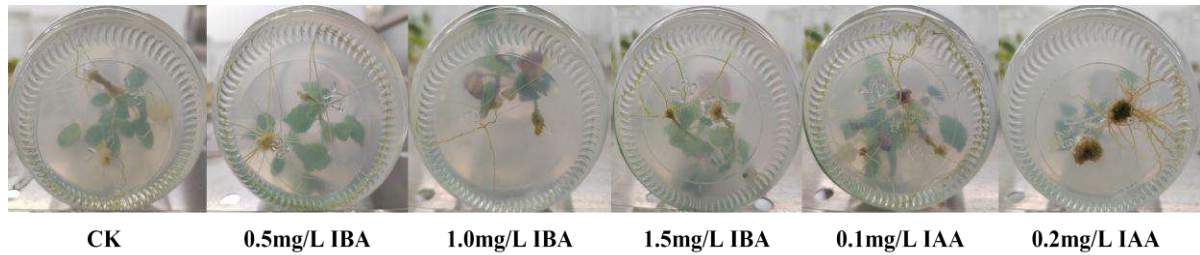


Fig. 2 Rooting situation of different PGRs

3.3 Adventitious buds and seedling formation induced from *W. indica* hypocotyls

The hypocotyls of *W. indica* were inoculated into MS + 0.1 mg / L 6-BA induction medium. After 15 days of culture, the hypocotyls began to expand, and yellow callus began to form from both sides to the middle (Fig. 3A). After 40 days of culture, the surface of the callus differentiated into a robust adventitious bud cluster (Fig. 3B). After 60 days of culture, it further developed into a seedling with adventitious buds and adventitious roots (Fig. 3C).

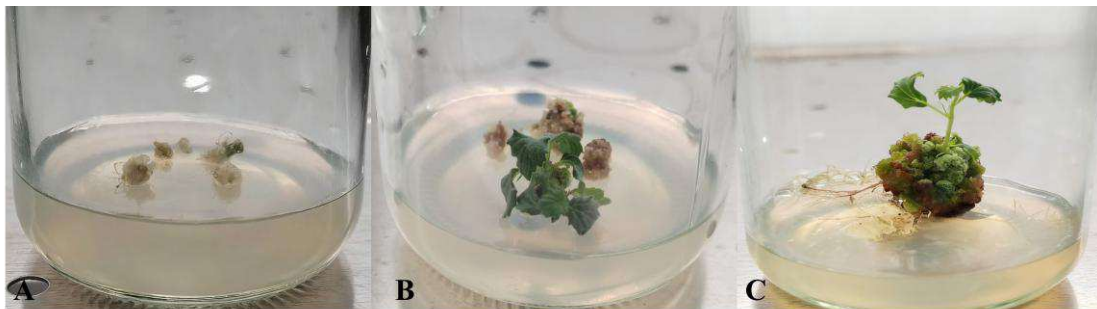


Fig. 3 Adventitious buds induced by hypocotyls

A: Adventitious buds were induced from hypocotyls for 15 days; B: Adventitious buds were induced from hypocotyls for 40 days; C: Adventitious buds and roots were induced from hypocotyls for 60 days.

3.4 Adventitious buds and roots induction from leaves of *W. indica* sterile seedlings

Sterile leaves of *W. indica* were inoculated onto callus induction medium (MS basal medium supplemented with 1.0 mg/L 6-BA, 0.2 mg/L NAA, and 1.0 mg/L 2,4-D). After 15 days, induced callus appeared as yellowish-green friable masses (Fig. 4A), with a 100% induction rate. These callus were transferred to proliferation medium (MS basal medium supplemented with 0.1 mg/L NAA; Fig. 4B). Following 20 days of culture, significant callus proliferation was observed (Fig. 4C), maintaining yellowish-green friable morphology.

Proliferating callus was transferred to differentiation medium (MS basal medium

supplemented with 1.0 mg/L 6-BA, 0.2 mg/L NAA, and 1.0 mg/L 2,4-D). Adventitious root initiation was observed at 10 days (Fig. 5A), with significant proliferation occurring between 20-30 days (Fig. 5B, C).



Fig.4 Callus induced and proliferated from leaves of *W. indica* sterile seedlings

A: Induced callus ; B: Inoculation of subculture callus ; C: Proliferated callus.

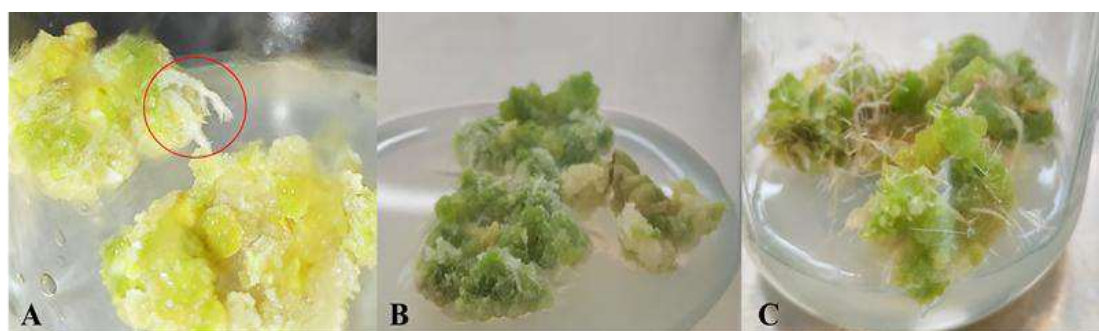


Fig. 5 Adventitious roots induced by callus

A: Adventitious roots induced from callus for 10 days; B: Adventitious roots induced from callus for 20 days; C: Adventitious roots induced from callus for 30 days.

3.5 Establishment of adventitious root suspension culture system of *W. indica*

As presented in Table 8, the relative proliferation multiple of adventitious roots initially increased and then decreased with increasing NAA concentration. When the NAA concentration was 1.0 mg/L, the relative proliferation multiple reached 5.03-fold, and the adventitious roots grew vigorously. Fig.6 shown the suspension culture of adventitious roots with different NAA concentrations. The adventitious roots gathered into clusters and new adventitious roots were generated during the suspension culture. In summary, the suitable medium for adventitious roots of *W. indica* was MS basal medium containing 30 g/L sucrose and 1.0 mg/L NAA.

In this study, the effect of inoculation amount on the kinetics of adventitious root suspension culture was investigated. The results showed that there was an optimal range for the inoculation amount, as presented in Table 9. After 30 days of culture in a 100 mL medium, the biomass of adventitious roots exhibited a distinct single-peak trend

with increasing inoculation amount. The optimal inoculation amount was 1%, at which the relative proliferation rate (4.09 times) was significantly higher than that in other groups.

Table 8 Effects of different NAA concentrations on adventitious root suspension culture

NAA (mg/L)	Initial inoculation fresh weight (g)	30d adventitious root fresh weight (g)	Net income (g)	Relative proliferation multiple
0	0.45	0.79±0.09d	0.34±0.09d	0.76±0.2d
0.1	0.45	1.77±0.17c	1.32±0.17c	2.93±0.37c
0.5	0.45	2.12±0.05b	1.67±0.05b	3.72±0.11b
1.0	0.45	2.71±0.09a	2.26±0.09a	5.03±0.2a
2.0	0.45	2.07±0.04b	1.62±0.04b	3.6±0.08b

Note: Different lowercase letters indicate significant differences ($P < 0.05$).



CK 0.1 mg/L NAA 0.5 mg/L NAA 1.0 mg/L NAA 2.0 mg/L NAA

Fig. 6 Effects of different NAA concentrations on adventitious root suspension culture.

Table 9 Effects of different inoculation amounts on adventitious root suspension culture

Serial Number	Initial inoculation(g)	30d adventitious root fresh weight (g)	Net income (g)	Relative proliferation multiple
1	0.5	2.42±0.35d	1.92±0.35bc	3.83±0.70a
2	1	5.09±0.17b	4.09±0.17a	4.09±0.17a
3	1.5	5.65±0.45a	4.15±0.45a	2.76±0.30b
4	2	4.29±0.17c	2.29±0.17b	1.15±0.08c
5	2.5	4.15±0.21c	1.66±0.20c	0.66±0.08c

Note : Different lowercase letters indicate significant differences ($P < 0.05$).

3.6 The *W. indica* adventitious root growth curve results in suspension culture

As presented in Fig.7, the growth curve of adventitious roots could be roughly divided into three stages. The 0-5d was the lag phase, at which time, the cells adapted to the new environment and grew slowly. The 5-30d was the exponential phase during

which time there was a fixed and high growth rate. During this phase, the nutrition and space in the medium did not reach the environmental capacity, the growth rate of adventitious roots continued to increase, the biomass also accelerated, and the overall growth curve was J-shaped. After 30 days, the cell growth rate slowed down, and the cell biomass reached the maximum at 35 days. After that, the cells directly entered the decline phase. At this time, the nutrients in the culture medium were depleted, the toxins were accumulated, and the cell growth was inhibited. Consequently, the best subculture time of *W. indica* adventitious root suspension culture was 30 days after inoculation.

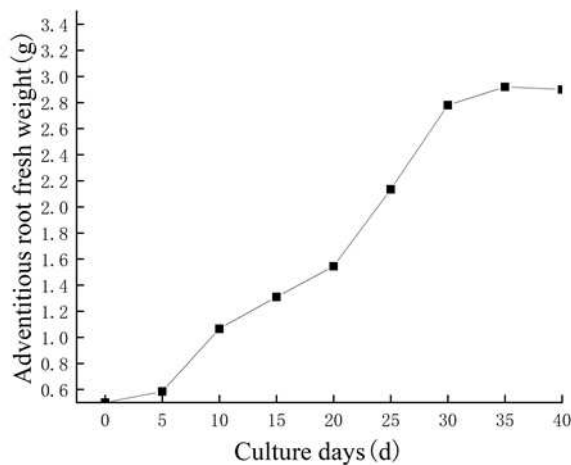


Fig. 7 Growth curve of adventitious root suspension culture

3.7 Nematode chemotaxis results of compounds from *W. indica*

As presented in Fig 8, 10 of the 12 compounds extracted from *W. indica* had a repellent effect on J2s of *M. incognita* at a concentration of 10 mg/L. Compound 9 had an attractive effect, and compound 11 had neither repellent nor attractive effects on J2s. Among them, compound 8 (Trans-tiliroside) had the best repellent effect, with a chemotaxis index of 0.22. Compound 7 ((+)-Propacin) and Compound 12 (Antidesmone) also had good repellent effects, with a chemotaxis index of 0.24 and 0.41, separately. (Note: CI > 1 indicates attraction, and CI < 1 indicates repellence). As presented in Fig 9, the repellent activities of two compounds against *M. incognita* were evaluated at five different concentrations. The results indicated that trans-tiliroside exhibited the strongest repellent activity at 10 mg/L, with a chemotaxis index of 0.29, whereas antidesmone showed the highest activity at 1 mg/L, with a chemotaxis index of 0.26. Because both compounds showed good repellent effects, they were selected for subsequent detection.

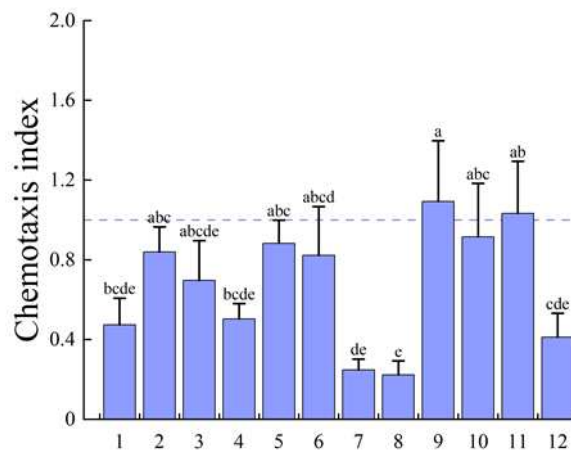


Fig 8 Chemotaxis index of 12 compounds against *M. incognita* at 10 mg / L concentration

Note: Different lowercase letters indicate significant differences ($P < 0.05$).

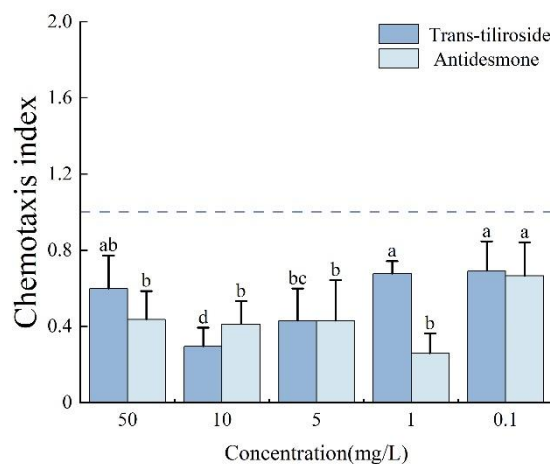


Fig.9 Chemotaxis index of trans-tiliroside and antidesmone to *M. incognita* at different concentrations

Note: Different lowercase letters indicate significant differences ($P < 0.05$).

3.8 Content detection results of bioactive compounds in different tissues of *W. indica*

As presented in Fig 10, this study explored the influence of different culture conditions on the content of antidesmone in the adventitious roots of *W. indica* and detected the antidesmone content in roots, stems, and leaves. The antidesmone content results in root (Fig. 10A) indicated that the liquid suspension culture yielded the highest

level, reaching 1.39% of dry weight (the same unit applies below). Under tissue culture conditions, the antidesmone content in root of the treatment with 0.05 mg/L 6-BA was significantly lower as compared the group without 6-BA, suggesting that 0.05 mg/L 6-BA inhibits the accumulation of antidesmone in the roots of *W. indica*. Regarding antidesmone content in stem (Fig. 10B), tissue-cultured plants exhibited a significantly higher level (1.01%) compared to wild-type plants. However, no significant difference was observed between the treatments with and without 0.05 mg/L 6-BA, indicating that 6-BA has no significant effect on antidesmone accumulation in the stems. For antidesmone content in leaf (Fig. 10C), tissue-cultured plants also showed a significantly higher level (0.78%) than wild-type plants. Similarly, no significant difference was found between the treatments with and without 0.05 mg/L 6-BA, demonstrating that 6-BA also has no significant impact on antidesmone accumulation in the leaves.

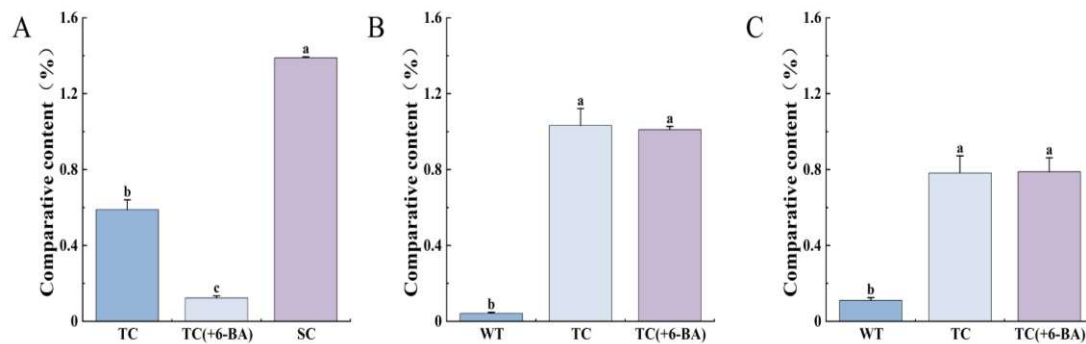


Fig. 10 Relative content of antidesmone in *W. indica* under different cultivation methods

A: The content of antidesmone in the root of *W. indica*; B: The content of antidesmone in the stem of *W. indica*; C: The content of antidesmone in the leaf of *W. indica*. TC is tissue culture, SC is suspension culture, WT is wild type. Different lowercase letters indicate significant differences ($P < 0.05$).

As presented in Fig 11, this study applied different culture conditions to *W. indica* and detected the trans-tiliroside content (extracted with methanol) in its roots, stems, and leaves. The results showed that trans-tiliroside was not detected in the roots under any culture condition. Analysis of stem trans-tiliroside content revealed that tissue-cultured plants contained 0.06% (Fig. 11A), which was significantly lower than that in wild-type plants. However, the addition of 0.05 mg/L 6-BA increased the trans-tiliroside content in the stems to 0.14%, higher than that in the group without 6-BA, indicating that 6-BA promotes the accumulation of trans-tiliroside in the stems. For trans-tiliroside content in leaf (Fig. 11B), tissue-cultured plants also showed a significantly lower level

(0.07%) compared to wild-type plants. Similarly, the application of 0.05 mg/L 6-BA increased the trans-tiliroside content in the leaves to 0.15%, higher as compared with untreated group, suggesting that 6-BA also enhances trans-tiliroside accumulation in the leaves.

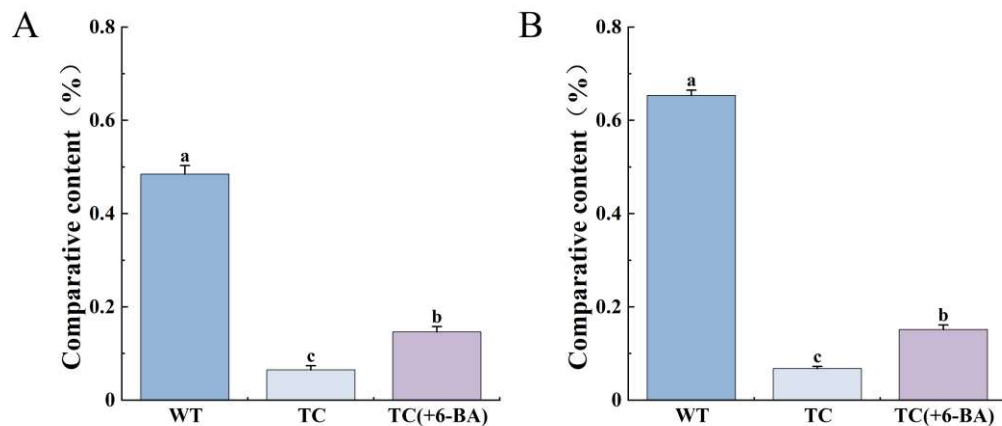


Fig. 11 Relative content of trans-tiliroside in *W. indica* under different cultivation methods

A: The content of trans-tiliroside in the stem of *W. indica*; B: The content of trans-tiliroside in the leaf of *W. indica*. TC is Tissue culture , WT is Wild type. Different lowercase letters indicate significant differences ($P < 0.05$).

4. Discussions

The disinfection of exogenous organisms is the first step in obtaining a sterile plant. Commonly used disinfecting agents in plant tissue culture include ethanol, mercuric chloride, and sodium hypochlorite (Ahmadpoor F et al,2022). Mercuric chloride is generally considered the most effective disinfectant; however, it is highly toxic and causes environmental pollution. Report found that the use of sodium hypochlorite, ethanol and other less harmful reagents for a certain time of treatment, can also achieve better disinfection effect (Pavese V et al,2022). In this study, *W. indica* seeds were used as explants. Surface sterilization with 75% ethanol for 30 seconds followed by 15% NaClO for 15 minutes resulted in zero explant contamination and a high seed germination rate (Table 5). The proliferation of sterile seedlings requires induction and regulation by plant hormones. Cytokinins and auxins are key factors affecting axillary bud formation in sterile seedlings (Wen Shu-Sheng et al,2020). Hormones commonly used for woody plants include 6-BA, IAA, and IBA (Khamushi M et al,2019). As optimal hormone types and concentrations vary among plant species, this study identified MS medium supplemented with 0.05 mg/L 6-BA as most suitable for the

proliferation of *W. indica* sterile seedlings, yielding a proliferation coefficient of 9.2 and promoting vigorous growth (Table 6).

Rooting culture is a critical stage in tissue culture, significantly influencing transplant survival rates by the number and quality of roots formed (Yu G et al,2019). During root induction, auxins play a vital role in promoting the development of lateral and adventitious roots (Long Y et al,2022; Duan X et al,2023). This study tested media containing specific concentrations of IAA and IBA. Results indicated that high concentrations of both IBA and IAA inhibited root formation, while MS medium supplemented with 0.1 mg/L IAA was optimal for rooting in *W. indica* sterile seedlings (Table 7, Fig. 2). Using plant tissue culture to regenerate the entire plant is equally important (Ozyigit et al,2022). The source of callus material has a very significant impact on the induction of tissue culture. In this study, an adventitious buds and roots were successfully induced from *W. indica* hypocotyls, and finally were induced into a whole seedling (Fig. 3). However, leaves of *W. indica* sterile seedlings were only induced into adventitious buds and roots (Fig. 4, 5).

Adventitious root suspension culture is influenced by numerous factors, among which exogenous hormones play a key role in proliferation (Alwakil N H et al,2022). Testing various concentrations of NAA in MS liquid medium revealed that high NAA concentrations inhibited adventitious root growth. The optimal medium for *W. indica* adventitious root suspension culture was MS supplemented with 30 g/L sucrose and 1.0 mg/L NAA (Table 8). Fig.6 shown the best suspension culture of adventitious roots with 1.0 mg/L NAA concentrations. The adventitious roots gathered into clusters and new adventitious roots were generated in the MS basal medium containing 30 g/L sucrose and 1.0 mg/L NAA. The effect of inoculation amount on the adventitious root suspension culture was investigated. Results showed that there was an optimal range for the inoculation amount, as amounts that were either too high or too low were not conducive to biomass accumulation. After 30 days of culture in a 100 mL medium, the biomass of adventitious roots exhibited the optimal inoculation amount was 1%, at which the relative proliferation rate (4.09 times) was significantly higher than that in other groups (Table 9). This phenomenon reveals the dual effects of the inoculation amount: an appropriate amount enables the culture system to achieve an optimal balance between nutrient supply and dissolved oxygen levels, leading to efficient growth; an excessive amount inhibits growth due to the rapid depletion of resources, while an insufficient amount reduces production efficiency because of a prolonged

culture cycle.

Different culture conditions are crucial for regulating the synthesis of plant secondary metabolites (Liu P et al,2025). Adventitious root suspension culture technology offers significant advantages, including rapid growth, stable metabolic profiles, and independence from seasonal and geographical constraints, and shows great potential for the industrial production of active compounds from medicinal plants (Hussain M J,2022). For *W. indica*, studies demonstrated that the content of the target alkaloid antidesmone in adventitious roots obtained via suspension culture was significantly higher than in wild type (Fig. 10A), confirming the effectiveness of this technique for enhancing specific secondary metabolite production.

The regulatory role of exogenous hormones on secondary metabolism often exhibits organ specificity (Wang Z et al,2025). The cytokinin 6-BA showed such a dual effect on antidesmone accumulation in *W. indica*. It significantly inhibited synthesis and accumulation in the roots but had no notable effect on antidesmone content in stems and leaves (Fig.10). Notably, conventional tissue culture conditions alone effectively induced antidesmone synthesis in stems and leaves, achieving levels significantly higher than those in wild-type plants (Fig. 10B, C), highlighting the potential of tissue culture for targeted compound production. Furthermore, the tissue culture environment somewhat suppressed the synthesis of trans-tiliroside in the stems and leaves of *W. indica*. However, this inhibitory effect was reversible through the targeted application of exogenous hormones. The addition of a low concentration (0.05 mg/L) of 6-BA to the culture medium effectively alleviated the suppression and significantly promoted the accumulation of trans-tiliroside in both stems and leaves (Fig.11). This phenomenon underscores the critical role of plant hormones in the fine-tuned regulation of distinct metabolic pathways.

Author contributions

MT and XL: methodology, experimental design, formal analysis, data interpretation and writing the original draft; RS, SW, ZH, DL: Conceptualization, experiment design, funding acquisition, supervision, manuscript editing; RS, SW, YG and SA: editing and improvement of the manuscript. The manuscript was read and approved by all authors.

Data availability

The original contributions presented in the study are included in the article/supplementary material.

Compliance with Ethical Standards

555 Research is not involving Human Participants or Animals, has no ethical issues.

556 **Declarations**

557 Conflict of Interest The authors declare no conflict of interest that could have
558 influenced the work reported in this paper.

559

Reference

- Ahmadpoor F, Zare N, Asghari R & Sheikhzadeh P (2022) Sterilization protocols and the effect of PGRs on callus induction and secondary metabolites production in in vitro cultures *Melia azedarach* L. *AMB Express* 12(1):3 doi:10.1186/s13568-022-01343-8
- Ahmar S, Gill RA, Jung KH, Faheem A, Qasim MU, Mubeen M & Zhou W (2020) Conventional and Molecular Techniques from Simple Breeding to Speed Breeding in Crop Plants: Recent Advances and Future Outlook. *Int J Mol Sci* 21(7) doi:10.3390/ijms21072590
- Alwakil NH, Mohamad Annuar MS & Jalil M (2022) Synergistic Effects of PGRs and Elicitors on alpha-Humulene and Zerumbone Production in *Zingiber zerumbet* Smith Adventitious Root Cultures. *Molecules* 27(15) doi:10.3390/molecules27154744
- Azlay L, El Boukhari MEM, Mayad EH & Barakate M (2022) Biological management of root-knot nematodes (*Meloidogyne* spp.): a review. *Organic Agriculture* 13(1):99-117 doi:10.1007/s13165-022-00417-y
- Babich O, Sukhikh S, Pungin A, Ivanova S, Asyakina L & Prosekov A (2020) Modern Trends in the In Vitro Production and Use of Callus, Suspension Cells and Root Cultures of Medicinal Plants. *Molecules* 25(24) doi:10.3390/molecules25245805
- Beeman AQ, Njus ZL, Pandey S & Tylka GL (2016) Chip Technologies for Screening Chemical and Biological Agents Against Plant-Parasitic Nematodes. *Phytopathology* 106(12):1563-1571 doi:10.1094/PHYTO-06-16-0224-R
- Dong L, Li X, Huang C, Lu Q, Li B, Yao Y, Liu T & Zuo Y (2018) Reduced *Meloidogyne incognita* infection of tomato in the presence of castor and the involvement of fatty acids. *Scientia Horticulturae* 237:169-175 doi:10.1016/j.scienta.2018.03.066
- Duan X, Chen L, Liu Y, Chen H, Wang F & Hu Y (2023) Integrated physicochemical, hormonal, and transcriptomic analysis reveals the underlying mechanism of callus formation in *Pinellia ternata* hydroponic cuttings. *Front Plant Sci* 14:1189499 doi:10.3389/fpls.2023.1189499
- Ferreira R, Maleita C, Fonseca L, Esteves I, Sousa-Ferreira I, Cabrera R & Castilho P (2025) Chemical Screening and Nematicidal Activity of Essential Oils from Macaronesian and Mediterranean Plants for Controlling Plant-Parasitic Nematodes. *Plants* 14(3) doi:10.3390/plants14030337
- Gelfand LL & Sorokin AA (1985) Gastrointestinal forms of salmonellosis caused by *S. infantis* and *S. typhimurium*. *Kazan medical journal*
- Gulzar B, Mujib A, Qadir Malik M, Mamgain J, Syeed R & Zafar N (2020) Plant tissue culture: agriculture and industrial applications Transgenic Technology Based Value Addition in Plant Biotechnology. p 25-49
- Hussain MJ, Abbas Y, Nazli N, Fatima S, Drouet S, Hano C & Abbasi BH (2022) Root Cultures, a Boon for the Production of Valuable Compounds: A Comparative Review. *Plants (Basel)* 11(3) doi:10.3390/plants11030439
- Jang JY, Le Dang Q, Choi GJ, Park HW & Kim JC (2019) Control of root-knot nematodes using *Waltheria indica* producing 4-quinolone alkaloids. *Pest Manag Sci* 75(8):2264-2270 doi:10.1002/ps.5363
- Kavitha TR, Prajwal B, Sunitha TR, Prema GU, Kranti KVVS, Suneetha C & Priya KT (2025) *Meloidogyne* Compendium of Phytopathogenic Microbes in Agro-Ecology p479-503
- Khamushi M, Dehestani-Ardakani M, Zarei A & Kamali Aliabad K (2019) An efficient protocol for

604 micropropagation of old cypress of Abarkuh (*Cupressus sempervirens* var. *horizontalis*
605 [Mill.]) under in vitro condition. *Plant Cell, Tissue and Organ Culture (PCTOC)*
606 138(3):597-601 doi:10.1007/s11240-019-01645-z

607 Khan MR (2023) Nematode Pests of Agricultural Crops, a Global Overview Novel Biological and
608 Biotechnological Applications in Plant Nematode Management. p 3-45

609 Khezri M, Asghari-Zakaria R & Zare N (2024) Plant Cell and Tissue Culture: Propagation,
610 Improvement, and Conservation of Medicinal Plants Biosynthesis of Natural Products in
611 Plants. p 267-291

612 Kihika R, Murungi LK, Coyne D, Ng'ang'a M, Hassanali A, Teal PEA & Torto B (2017) Parasitic
613 nematode *Meloidogyne incognita* interactions with different *Capsicum annum* cultivars
614 reveal the chemical constituents modulating root herbivory. *Sci Rep* 7(1):2903
615 doi:10.1038/s41598-017-02379-8

616 Kirwa HK, Murungi LK, Beck JJ & Torto B (2018) Elicitation of Differential Responses in the
617 Root-Knot Nematode *Meloidogyne incognita* to Tomato Root Exudate Cytokinin,
618 Flavonoids, and Alkaloids. *J Agric Food Chem* 66(43):11291-11300
619 doi:10.1021/acs.jafc.8b05101

620 Kuang MS, Liu TT, Wu HB, Lan HP, Wen YX, Wu HB & Li XM (2020) Constituents Leached by
621 Tomato Seeds Regulate the Behavior of Root-Knot Nematodes and Their Antifungal
622 Effects against Seed-Borne Fungi. *J Agric Food Chem* 68(34):9061-9069
623 doi:10.1021/acs.jafc.0c01797

624 La S, Li J, Ma S, Liu X, Gao L & Tian Y (2024) Protective role of native root-associated bacterial
625 consortium against root-knot nematode infection in susceptible plants. *Nat Commun*
626 15(1):6723 doi:10.1038/s41467-024-51073-7

627 Liang C, Yang L, Shao Y, Zhu X, Zhao H, Chen B, Song W, Song X, Ding X & Sun R (2019) Broad-
628 spectrum antifungal activity of dichloromethane extract of *Waltheria indica* stems and
629 isolated compounds. *Industrial Crops and Products* 142 doi:10.1016/j.indcrop.2019.111855

630 Liu P, Feng W, Yang H, Yu G, Shang E, Zhang S, Yan H & Duan JA (2025) Untargeted metabolomics
631 and functional analyses reveal that the secondary metabolite quinic acid associates with
632 *Angelica sinensis* flowering. *BMC Plant Biol* 25(1):72 doi:10.1186/s12870-025-06101-z

633 Long Y, Yang Y, Pan G & Shen Y (2022) New Insights Into Tissue Culture Plant-Regeneration
634 Mechanisms. *Front Plant Sci* 13:926752 doi:10.3389/fpls.2022.926752

635 Manoj Kumar Tripathi ST, Niraj Tripathi, Gyanendra Tiwari, Deepa Bhatt, Megha Vibhute, ...
636 Sharad Tiwari (2021) Plant Tissue Culture Techniques for Conservation of Biodiversity of
637 Some Plants Appropriate for Propagation in Degraded and Temperate Areas. *Current Topics*
638 *in Agricultural Sciences* Vol. 4, 30–60. doi:doi.org/10.9734/bpi/ctas/v4/2119C

639 Nirmala C & Sridevi M (2021) Ethnobotanical, phytochemistry, and pharmacological property of
640 *Waltheria Indica* Linn. *Future Journal of Pharmaceutical Sciences* 7(1)
641 doi:10.1186/s43094-020-00174-3

642 Ozyigit I I, Dogan I, Hocaoglu-Ozyigit A, et al (2023) Production of secondary metabolites using
643 tissue culture-based biotechnological applications. *Frontiers in Plant Science*, 14: 1132555.

644 Pavese V, Ruffa P, Abba S, Costa RL, Corredoira E, Silvestri C, Torello Marinoni D & Botta R
645 (2022) An In Vitro Protocol for Propagating *Castanea sativa* Italian Cultivars. *Plants (Basel)*
646 11(23) doi:10.3390/plants11233308

647 Sarma MK, Sharma AA, Samantara K & Wani SH (2023) In Vitro Techniques in Plant Breeding

648 Advanced Crop Improvement, Volume 1. p 185-215
 649 Sehgal D & Khan T (2020) Plant Tissue Culture: Beyond Being a Tool for Genetic Engineering
 650 Environmental Microbiology and Biotechnology. p 175-200
 651 Su W, Xu M, Radani Y & Yang L (2023) Technological Development and Application of Plant
 652 Genetic Transformation. Int J Mol Sci 24(13) doi:10.3390/ijms241310646
 653 Swaruparani N & Shanmugam H (2024) Molecular mechanisms and phytomolecules as source of
 654 resistance for sustainable management of root knot nematode infestations in horticultural
 655 crops: A review. Physiological and Molecular Plant Pathology 133
 656 doi:10.1016/j.pmpp.2024.102354
 657 Terra WC, Lopes de Paula L, de Brum D, Campos VP, de Oliveira DF & De Souza JT (2023)
 658 Chemotaxis in Root-Knot Nematodes Root-Galling Disease of Vegetable Plants. p 85-115
 659 Tsai AY, Iwamoto Y, Tsumuraya Y, Oota M, Konishi T, Ito S, Kotake T, Ishikawa H & Sawa S (2021)
 660 Root-knot nematode chemotaxis is positively regulated by l-galactose sidechains of
 661 mucilage carbohydrate rhamnogalacturonan-I. Sci Adv 7(27) doi:10.1126/sciadv.abh4182
 662 Wang Z, Yun Q, Hu J, Wei Z, Feng D, Li N, Xu H, Fu L, Wang Z, Li S, Liu F, Wang Y, Cong B &
 663 Wang B (2025) Spatiotemporal dynamics of benzylisoquinoline alkaloid gene expression
 664 and co-expression networks during Papaver Somniferum developmental stages. Sci Rep
 665 15(1):27406 doi:10.1038/s41598-025-11942-7
 666 WEI Y, Xie J, Wang S, DING X, YANG Z, BAI Y, YAO Y & SUN R (2023) Establishment and
 667 application of quantitative evaluation method for the chemotaxis of *Meloidogyne incognita*.
 668 Chinese Journal of Pesticide Science 25(5):1068-1075
 669 Wen S-S, Chen L & Tian R-N (2020) Micropropagation of tree peony (*Paeonia* sect. Moutan): A
 670 review. Plant Cell, Tissue and Organ Culture (PCTOC) 141(1):1-14 doi:10.1007/s11240-
 671 019-01747-8
 672 Yabre Z, Boly R, Ouedraogo R, Couliadiaty AGV, Somda GD, Semde R, Ouedraogo N & Youl ENH
 673 (2024) A review of the safety profile, antioxidant, anti-inflammatory, and bronchorelaxant
 674 activities of *Waltheria indica* Linn (Malvaceae): A potential antiasthmatic phytoedicine.
 675 Heliyon 10(12):e32402 doi:10.1016/j.heliyon.2024.e32402
 676 Yin F, Liu Q, Zhang B, Zhang X, He J, Xie J, Hu Z & Sun R (2021) Microemulsion preparation of
 677 *Waltheria indica* extracts and preliminary antifungal mechanism exploration. Industrial
 678 Crops and Products 172 doi:10.1016/j.indcrop.2021.114000
 679 Yu G, Wang J, Miao L, Xi M, Wang Q & Wang K (2019) Optimization of Mature Embryo-Based
 680 Tissue Culture and Agrobacterium-Mediated Transformation in Model Grass
 681 Brachypodium distachyon. Int J Mol Sci 20(21) doi:10.3390/ijms20215448
 682 Zhou Z, Wang T, Xu S, Li P, Xu Q & Su J (2025) Ultrasound-assisted extraction of phenolic
 683 compounds from *Waltheria indica* L.: purification, identification and bio-functional
 684 activities. Microchemical Journal 218 doi:10.1016/j.microc.2025.115333
 685 Zongo F, Ribaut C, Boumendjel A & Guissou I (2013) Botany, traditional uses, phytochemistry and
 686 pharmacology of *Waltheria indica* L. (syn. *Waltheria americana*): A review. Journal of
 687 Ethnopharmacology 148(1):14-26 doi:10.1016/j.jep.2013.03.080

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Appendix1105.docx](#)