

In vitro regeneration via indirect organogenesis from aseptic seedling-derived explants of the rare Tibetan medicinal shrub *Sibiraea angustata*

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Abstract

To conserve the germplasm resources of *S. angustata* and support its subsequent molecular biological research, this study aimed to establish efficient artificial propagation protocol. Using seeds of *S. angustata* as experimental materials, we investigated the effects of different medium formulations on seed germination, and further explored the impacts of various hormone ratios on its tissue culture via a complete factorial experimental design. The results showed that 1/4 MS was the optimal medium for both seed germination and seedling growth. Stem segments were more suitable as tissue culture explants than leaves. The optimal medium for stem segment callus induction was 1/4 MS + 1.0 mg·L⁻¹ 6-BA + 1.0 mg·L⁻¹ NAA (induction rate: 76.67%), for adventitious bud induction was 1/2 MS + 1.0 mg·L⁻¹ 6-BA + 0.1 mg·L⁻¹ NAA (induction rate: 83.33%), and for rooting induction was 1/4 MS + 1.0 mg·L⁻¹ IBA (average rooting rate: 80%; average root number: 6.83). This study successfully established a complete tissue culture and rapid propagation system for *S. angustata*, solving its rooting difficulty and providing technical support for the sustainable utilization, industrial seedling breeding, and introduction and domestication of this precious Tibetan medicinal plant.

Key message

The first complete establishment of a tissue culture regeneration system for a rare Tibetan medicinal plant of *Sibiraea angustata*.

Introduction

Tibetan medicine relies on a rich diversity of plant species endemic to the high-altitude Qinghai-Tibet Plateau. These plants have evolved under extreme environmental conditions, characterized by low temperatures, intense ultraviolet radiation, large diurnal temperature fluctuations, and infertile soils, and consequently synthesize a broad spectrum of bioactive secondary metabolites (Chen et al. 2018; Liang et al. 2022). Among these medicinal plants, *Sibiraea angustata*, commonly known as “liucha”, is a perennial woody shrub belonging to the genus *Sibiraea* Maxim. of the Rosaceae family. It is widely distributed across central and western China, naturally thriving at altitudes of 3000–4000 m on shrubby slopes and gravelly riverbanks, where it undergoes a prolonged dormancy period. Characterized by heliophily, high cold tolerance, and drought resistance, *S. angustata* is well-adapted to infertile habitats and exhibits broad soil adaptability, being capable of growing in both neutral and slightly acidic soil conditions (Ou 2020).

S. angustata has long been utilized in traditional Tibetan medicine owing to its valuable medicinal properties, and its leaves are commonly prepared as tea or utilized in folk medicine in high-altitude regions (Zhang 2022). Pharmacological studies have demonstrated its diverse bioactivities, including hypolipidemic (Liu et al. 2022; Liu et al. 2020), antitumor (Cheng et al. 2016; Luo and Liu 2007), hepatoprotective (Wang et al. 2011; Yao et al. 2010), and antioxidant effects (Yang et al. 2022).

Furthermore, due to its elegant appearance and an extended flowering period of 3–4 months, *S. angustata* also represents a highly promising ornamental species (Zhang 2006).

Owing to their unique habitats, Tibetan medicinal plants are frequently characterized by slow growth, scarce resource availability, and patchy species distribution (Liang et al. 2022). In recent years, rising demand for *S. angustata* has intensified wild collection, resulting in a sharp decline in natural populations. Thus, the conservation and sustainable use of its germplasm resources have become a matter of great urgency. Presently, resource conservation of *S. angustata* is achieved mainly through artificial seeding and division propagation (Zhang 2006). However, such conventional propagation methods are limited by lengthy breeding cycles and strict environmental requirements, failing to substantially mitigate resource scarcity.

Plant tissue culture constitutes a breakthrough for propagating traditional Chinese medicinal materials. Free from temporal and spatial constraints, it supports year-round production and is crucial for conserving medicinal plant resources (Zhang et al. 2023). Tissue culture has been successfully employed in various Tibetan medicinal plants, such as *Rheum tanguticum* (Yang et al. 2024; Yu et al. 2026), *Gentiana straminea* (Liu et al. 2006), *Saussurea involucrate* (Guo et al. 2007), and *Przewalskia tangutica* (Lei et al. 2015), supporting its utility for germplasm preservation and sustainable exploitation.

To date, research on the tissue culture of *S. angustata* remains limited both domestically and internationally. Current studies have only addressed callus induction and browning inhibition (Guo et al. 2018), and a complete regeneration system has not yet been developed. Accordingly, we herein used *S. angustata* as explants to screen the optimal medium for seed germination and investigate the effects of various plant hormone combinations on callus induction, adventitious bud differentiation, and rooting, which a reliable and complete tissue culture regeneration system was established for *S. angustata*. This study firstly provides a technical foundation for the germplasm conservation, large-scale propagation, introduction and domestication, and subsequent molecular biological research of *S. angustata*.

Materials and methods

Plant materials and growth conditions

S. angustata was provided by Linzhi Plateau Resources Technology Co., Ltd., and identified by Professor Li Bin from Jiangxi University of Traditional Chinese Medicine. Seeds of *S. angustata* were extracted from mature spikes and collected in Linzhi, Xizang Autonomous Region, China (29.65° N, 94.36° E). Fresh seeds were used immediately for germination assays, while the remaining seeds were stored at -20°C for subsequent experiments. Sterile seedlings were cultured in an incubator at 23°C under a 16 h light/8 h dark photoperiod with a light intensity of 2500 lux. By comparison, field-grown seedlings were cultivated at 25°C under the same photoperiod and light intensity.

Culture media

MS basal medium supplemented with 3% (w/v) sucrose and 0.7% (w/v) agar was used in all experiments. The pH of all media was adjusted to 5.8 ± 0.1 with 0.1 M NaOH or 0.1 M HCl prior to autoclaving at 121°C for 15 min. The plant growth regulators employed included 6-BA (Coolaber, CB33141680), NAA (Coolaber, CN332119100), IAA (Coolaber, CI331926100), and IBA (Coolaber, CI331727180). Stock solutions of these growth regulators were prepared following the manufacturer's instructions, filter-sterilized using a 0.22 µm membrane filter, and added to autoclaved medium after cooling to approximately 50°C.

Establishment of aseptic seedling

To break seed dormancy, fresh seeds of *S. angustata* were thoroughly rinsed with distilled water and then stratified at 4°C for 60 days to enhance germination. Following vernalization, seeds were surface-sterilized in 75% ethanol for 3 min, then treated with 2% sodium hypochlorite solution containing Tween 20 (Coolaber, CT32211301H) for 4 min. After each sterilant treatment, seeds were rinsed three times with sterile distilled water. Sterilized seeds were then germinated on MS medium (Phytotech, HRX0404041B) solidified with 0.7% (w/v) agar (Coolaber, CA341337200). To identify the optimal basal medium strength for seed germination, seeds were cultured on MS, 1/2 MS, and 1/4 MS media. All germination assays were performed in 100 mm × 15 mm petri dishes, with 40 seeds per dish and three replicates per treatment. Seed germination and root development were assessed after 15 days of culture.

Germination Rate (%) = (Number of germinated seeds / Number of inoculated seeds) × 100%

Average Root Length (cm) = Total root length measured / Total number of roots measured

Callus induction

Healthy two-month-old aseptic seedlings of *S. angustata* were selected. Stems and leaves were excised and used as explants for callus induction. Explants were inoculated onto 1/4 MS basal medium supplemented with different concentrations of 6-BA (0.5, 1.0, 1.5, 2.0 mg·L⁻¹) and NAA (0.5, 1.0, 1.5 mg·L⁻¹) to induce callus formation in utilization of a full factorial experimental design resulting in 12 treatment combinations. For each treatment, 10 stem explants and 15 leaf explants were inoculated, with three biological replicates per treatment. After 30 days of culture, the effects of different 6-BA and NAA combinations on callus induction were evaluated based on callus growth status and callus induction rates, to identify the optimal explant type and the best callus induction medium formulation.

Callus Induction Rate (%) = (Number of explants forming callus / Total number of explants inoculated) × 100%

Adventitious bud differentiation

Healthy, compact, and pale green calli induced from the stem segments cultured for one month were selected and transferred to adventitious bud differentiation 1/2 MS medium, supplemented with NAA at 0.1, 0.5, and 1.0 mg·L⁻¹ and 6-BA at 0.5, 1.0, 1.5, and 2.0 mg·L⁻¹. Each treatment consisted of 10 calli and was replicated three times in a full factorial design, yielding a total of 12 treatments. The adventitious bud differentiation rate was evaluated after 60 days of culture.

Adventitious Bud Differentiation Rate (%) = (Number of calli forming adventitious buds / Total number of calli transferred) ×100%

Root Induction

Adventitious buds with a height of 2–3 cm and 3–4 true leaves were selected and transferred to rooting medium to induce root formation. The rooting medium 1/4 MS medium supplemented with different concentrations (0, 0.1, 0.5, 1.0 mg·L⁻¹) of the auxins IAA, IBA, or NAA. Each treatment was comprised of 10 adventitious buds, with three biological replicates. The rooting status was recorded after 45 days, including rooting rate and number of roots per seedling. The optimal rooting medium was selected based on comprehensive evaluation of these indicators.

Rooting Rate (%) = (Number of adventitious buds forming roots / Total number of transferred adventitious buds) ×100%

Average Root Number = Total number of roots on rooted seedlings/ Number of rooted seedlings

Acclimatization and Transplanting

Rooted plantlets with a plant height of ≥ 2.0 cm were selected for acclimatization and transplantation. The rooted seedlings were partially uncapped and placed in the incubator for 3 days to acclimatize them to the external environment. After the seedlings were stabilized, they were gently taken out of the flasks, and the residual medium attached to the roots was washed off carefully. Subsequently, the seedlings were soaked in a 0.1% carbendazim solution for 10 minutes to prevent fungal infection. After soaking, the seedlings were transplanted into seedling pots filled with a mixed substrate consisting of vermiculite and nutrient soil at a ratio of 2:1 (v/v). The transplanted seedlings were covered with plastic film to maintain appropriate humidity for 7 days. Each treatment consisted of 10 plants with three biological replicates. The survival rate of the transplanted seedlings was recorded 30 days after transplantation.

The Survival Rate (%) = (Number of surviving transplanted seedlings/ Number of transplanted seedlings) ×100%

Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD) of three biological replicates. Data analysis was performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Multiple samples were

several completely randomized design one-way ANOVA, and pairwise comparisons were determined using Duncan's multiple range test at a significance level of $P < 0.05$. Graphs were generated using GraphPad Prism 10 software.

Results

Seed dormancy breaking and germination of *S. angustata*

Cold stratification at 4°C for 60 days effectively broke seed dormancy in *S. angustata*, achieving a germination rate exceeding 90%. For most species in the Rosaceae family, MS medium is routinely employed for in seed germination. To identify the optimal inorganic salt concentration in MS medium, forty seeds of *S. angustata* were cultured on three strengths of MS basal medium of 1/4, 1/2, and MS. The mean germination percentages were 55%, 55%, and 62.5% on 1/4, 1/2, and MS media, respectively (Fig. 1A). No significant differences in germination rate were detected among the three media, suggesting that basal salt concentration exerted only a minor influence on seed germination. In contrast, root development was strongly affected by medium strength. Seedlings grown on 1/4 MS produced significantly longer roots (average 0.93 cm) than those on 1/2 MS (0.57 cm) and MS (0.23 cm) (Fig. 1B). Furthermore, root morphology varied considerably, where seedlings on 1/4 MS developed longer and healthier root systems, whereas those on 1/2 and MS displayed shorter, blackened roots (Fig. 1C).

Survival rates also differed depending on medium strength. Seedlings cultured on 1/2 and MS medium exhibited slow growth and gradually died within one month, with complete mortality recorded after two months. By contrast, all seedlings grown on 1/4 MS medium remained viable and grew steadily over the two-month culture period (Fig. 1C). Accordingly, 1/4 MS medium was determined to be the optimal basal medium for seed germination and subsequent aseptic seedlings establishment of *S. angustata*.

(A) Effects of different media on seed germination of *S. angustata*; (B) Effects of different media on root length of *S. angustata*, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$; (C) The growth of 15-day seedlings cultured on different media, scale bar = 1 cm.

Explant selection of *S. angustata*

Two types of explants (leaves and stem segments) were evaluated for callus induction. Callus initiation occurred approximately 10 days after inoculation in stem segments, whereas leaf explants required about 20 days to form visible calli. Furthermore, stem segments exhibited significantly higher callus induction efficiency than leaf explants. Calli derived from stem segments were bright green and compact in texture, proliferated vigorously, and were capable of further adventitious bud differentiation (Fig. 2B). In contrast, calli induced from leaf explants were yellowish-green and generally loose in structure, and were highly susceptible to browning and necrosis during subsequent cultivation (Fig. 2C). Collectively, comparative analysis demonstrated that stem segments were more suitable and responsive explants for the tissue culture of *S. angustata*.

(A) Aseptic seedlings grown for 60 days, scale bar = 1 cm; (B) Callus induced from stem segments, scale bar = 1 cm; (C) Callus induced from leaf explants, scale bar = 1 mm; (D) The induction rates of stem segments under various concentrations of NAA and 6-BA; (E) The induction rates of leaves under diverse concentrations of NAA and 6-BA, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$.

Callus induction and adventitious bud differentiation of *S. angustata*

The combination of exogenous plant growth regulators significantly influenced callus induction and adventitious bud differentiation. For callus induction, a higher concentration of NAA ($1.0 \text{ mg}\cdot\text{L}^{-1}$) combined with 6-BA in the range of $0.5\text{--}1.5 \text{ mg}\cdot\text{L}^{-1}$ resulted in a higher callus induction rate from stem explants, with callus induction rates exceeding 70%. At a given NAA concentration, callus induction rates in stem explants initially increased and subsequently declined with increasing 6-BA concentrations, with the shortest induction time and highest induction efficiency observed at $1.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA (Fig. 2D). By comparison, callus induction from leaf explants showed less consistent responses to exogenous plant growth regulators, which may be attributed to distinct hormonal requirements between explant types or to the severe browning that frequently occurred in leaf explants (Fig. 2E). Taken together, the optimal medium for callus induction from stem segments of *S. angustata* was identified as 1/4 MS medium supplemented with $1.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA and $1.0 \text{ mg}\cdot\text{L}^{-1}$ NAA.

For adventitious bud differentiation, thirty-day-old stem callus was used for adventitious bud induction, with 1/2 MS as the basal medium to test the effects of 6-BA ($0.5, 1.0, 1.5, 2.0 \text{ mg}\cdot\text{L}^{-1}$) and NAA ($0.1, 0.5, 1.0 \text{ mg}\cdot\text{L}^{-1}$) gradients. Adventitious bud formation was completely inhibited at 6-BA ($0.5\text{--}1.0 \text{ mg}\cdot\text{L}^{-1}$) and NAA ($1.0 \text{ mg}\cdot\text{L}^{-1}$) (Figs. 3). Unlike callus induction, a combination of a relatively high concentration of 6-BA and a low concentration of NAA was found to be optimal. Additionally, $6\text{-BA} \geq 1.5 \text{ mg}\cdot\text{L}^{-1}$ resulted in small, weak, and slow-growing adventitious buds. Consequently, the optimal medium for *S. angustata* adventitious bud induction is 1/2 MS + $1.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA + $0.1 \text{ mg}\cdot\text{L}^{-1}$ NAA, with a maximum induction rate of 83.33%.

(A) Clustered adventitious buds induced from callus, scale bar = 1 cm; (B) Effects of different concentrations of 6-BA and NAA on the adventitious bud induction rate of *S. angustata*, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$.

Root induction of *S. angustata*

Rooting of regenerated shoots presented a significant challenge in establishing the regeneration system. Firstly, we examined the effect of basal medium salt strength on in vitro rooting. Among MS, 1/2 and 1/4 MS media, 1/4 MS produced the most favorable rooting response, in agreement with the seed germination. Then, three auxins (NAA, IAA, and IBA) were evaluated for their ability to induce root formation from adventitious buds on 1/4 MS medium. Notably, adventitious buds failed to root and

eventually died on 1/4 MS medium lacking plant growth regulators. NAA did not induce rooting at any tested concentration. By contrast, both IAA and IBA promoted root formation, with initial root emergence observed at approximately one week of culture (Fig. 4A). Within the concentration range of 0.1-1.0 mg·L⁻¹, the rooting rate and root number increased with rising IBA concentration, whereas those of IAA initially increased and then decreased (Figs. 4B-C). Furthermore, IBA was significantly superior to IAA in terms of rooting efficiency and rooting percentage, producing more roots per shoot and more vigorous root systems. Collectively, the optimal rooting medium for *S. angustata* was determined as 1/4 MS supplemented with 1.0 mg·L⁻¹ IBA, which achieved a rooting rate of 80% and an average of 6.83 roots per shoot.

(A) Adventitious root formation of adventitious buds, scale bar = 1 cm; (B) The rooting rate after adventitious root induction from adventitious buds with different concentrations of IBA and IAA; (C) The root length after adventitious root induction from adventitious buds with different concentrations of IBA and IAA, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$.

Acclimatization and Transplantation of *S. angustata*

Regenerated plantlets with well-developed root systems were successfully acclimatized under greenhouse conditions. A substrate mixture of vermiculite and nutrient soil at a 2:1 ratio proved optimal for transplantation. After 30 days of transplantation, the tissue-cultured seedlings exhibited vigorous growth with bright green leaves and robust root systems (Fig. 5B), and the survival rate reached 56.7%. However, the overall survival rate remained relatively low following six months of acclimatization, indicating that further optimization of substrate composition and acclimatization protocols is required. Nonetheless, this study represents the first successful transplantation of *S. angustata* plantlets in the Nanchang region, demonstrating the feasibility of ex situ conservation and cultivation for the plateau-adapted species.

(A) *S. angustata* that reproduces by seeds, scale bar = 5 cm; (B) Tissue-cultured seedlings 60 days after transplantation, scale bar = 5 cm.

Discussion

As a valuable Tibetan medicinal plant, *S. angustata* has a restricted habitat and a long growth cycle. Unregulated exploitation without effective and timely conservation measures will inevitably lead to resource exhaustion. The production of sterile seedlings is not limited by natural geographical conditions, which enables research on the breeding techniques of *S. angustata* under ex situ conditions. To protect the germplasm resources and facilitate its subsequent molecular biological research, the present study successfully established sterile seedlings using seeds as explants and developed a complete tissue culture regeneration system for *S. angustata*.

Salt concentration and seed germination

MS medium is widely acknowledged as the most prevalent basal medium in plant tissue culture because of its high mineral salt content and well-balanced micronutrient profile (Wu et al. 2020). It is commonly used for seed germination and initial culture establishment in many Rosaceae plants (Chen et al. 2023; Wang et al. 2020). However, our results indicated that *S. angustata*, which is naturally adapted to nutrient-poor plateau soils, exhibited poor growth on MS medium, with severe stunting, root blackening, and high mortality (Fig. 1C). This observation likely reflects the natural adaptation of *S. angustata* to nutrient-poor habitats, as high nutrient concentrations may induce physiological stress and damage in the seedlings. The low-salt conditions of 1/4 MS medium better simulate the nutrient-poor natural soils of the Qinghai-Tibet Plateau. This result emphasizes the need to empirically determine the optimal medium salt concentration for each species, particularly for plants native to extreme habitats.

Seeds of Rosaceae species generally exhibit physiological dormancy, which can be broken by cold stratification (Liu et al. 2024). *S. angustata* is a plateau plant with strong cold tolerance. Compared with other Rosaceae plants, it has a longer dormancy period. Cold stratification at 4°C for 60 days was suitable for breaking seed dormancy, achieving a germination rate of over 90% (Fig. 1A).

Explant selection and browning control

Explant selection is a critical factor determining the success of plant tissue culture (Zhou et al. 2024). In this study, stem segments bearing axillary buds were superior to leaf explants in both shoot induction frequency and callus quality (Figs. 2). This observation agrees with previous findings on *S. angustata* (Guo et al. 2018), who showed that stem segments yield higher induction rates and less browning than leaves. The better performance of stem segments can be explained by their inherent meristematic tissues, which are more sensitive to exogenous growth regulators.

Browning resulting from phenolic oxidation in wounded explants represents a key limitation in tissue culture of medicinal plants (Bhat and Chandel 1991; Guo et al. 2018), mitigating browning effectively boosts culture success. The lower browning in stem segments compared to leaves may reflect differences in phenolic accumulation and tissue anatomy (Figs. 2B-C). Low-salt media suppressed browning, as previously documented in *Rubus chingii* (Wang and Jiang 2020) and *Sinopodophyllum hexandrum* (Xu et al. 2019). *S. angustata* exhibited much less browning on 1/4 MS than MS. Together, suitable explant selection and reliable browning control are indispensable for successful in vitro regeneration.

Plant growth regulator effects across culture stages

Plant growth regulator demands vary substantially across different stages of tissue culture. Here, a high NAA concentration ($1.0 \text{ mg} \cdot \text{L}^{-1}$) combined with moderate 6-BA ($0.5\text{--}1.5 \text{ mg} \cdot \text{L}^{-1}$) effectively facilitated callus induction (Figs. 2), in agreement with the general consensus that auxins are essential for callus formation (Ikeuchi et al. 2013).

Shoot differentiation required a high cytokinin-to-auxin ratio. The combination of $1.0 \text{ mg}\cdot\text{L}^{-1}$ 6-BA and $0.1 \text{ mg}\cdot\text{L}^{-1}$ NAA was optimal for adventitious bud formation in *S. angustata* (Figs. 3). This trend, in which elevated cytokinin and reduced auxin promote shoot proliferation, is well documented across Rosaceae plants, including apple rootstocks (Sedlak and Paprstein 2020) and roses (Lin 2019).

Rooting represented the most challenging stage of the regeneration system (Chen et al. 2023). The ineffectiveness of NAA for rooting, combined with the positive effects of IAA and IBA, demonstrates the species specificity of auxin action (Figs. 4). IBA is widely regarded as a reliable rooting compound for woody plants, owing to its higher stability and lower degradation rate compared with IAA (Sevik and Guney 2013). The better rooting performance of *S. angustata* with IBA aligns with reports on other Rosaceae plants, including *Malus* rootstocks (Yan et al. 2024) and *Sorbaria sorbifolia* (Yi et al. 2023). The lack of rooting response to NAA may result from its high activity and possible phytotoxicity at concentrations that work for other species.

Implications for conservation and sustainable utilization

The complete tissue culture regeneration system developed herein provides a solid foundation for the conservation and sustainable utilization of *S. angustata*. This protocol supports efficient year-round propagation free from the limitations of traditional methods (Ou 2020). It also enables mass production of clonal plantlets using minimal explants, which is highly beneficial for the germplasm preservation of this overharvested species (Liang et al. 2022). Moreover, the successful ex situ acclimatization in Nanchang confirms the feasibility of off-site cultivation, helping to reduce threats to wild resources.

However, the relatively low acclimatization survival rate indicates a need for further improvement. Conditions such as humidity, light intensity, and substrate may need to be optimized for local environments. Long-term observation is necessary to assess whether *S. angustata* plantlets can fully acclimate to Nanchang's subtropical climate, which is distinctly different from its original alpine habitat.

Abbreviations

MS Murashige and Skoog medium

6-BA 6-benzylaminopurine

IAA Indole-3-acetic acid

IBA Indole-3-butyric acid

NAA 1-naphthaleneacetic acid

Declarations

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Author contributions

MP conceived and designed the experiments; XY performed the experiments. MP wrote the manuscript; BL revised the manuscript. All the authors have read and approved the paper.

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Data availability

All data are available from the corresponding author upon reasonable request

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication

All authors approve and consent to publish this manuscript.

Ethics approval and consent to participate

The plant materials were collected in accordance with national relevant guidelines, and ethical approval was not required for this study.

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Figures

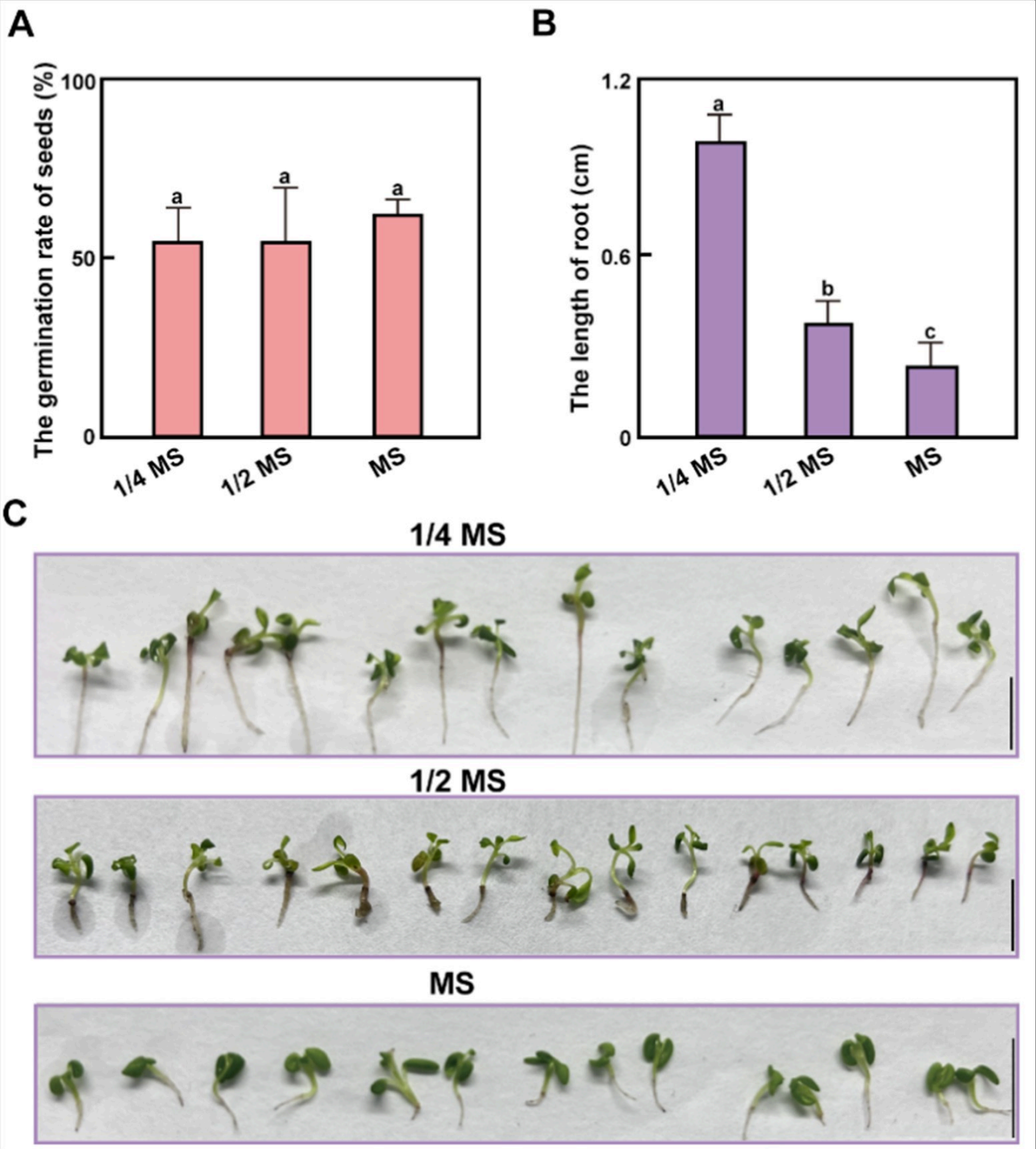


Figure 1

Growth of *S. angustata* seedlings in different media

(A) Effects of different media on seed germination of *S. angustata*; (B) Effects of different media on root length of *S. angustata*, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$; (C) The growth of 15-day seedlings cultured on different media, scale bar = 1 cm.

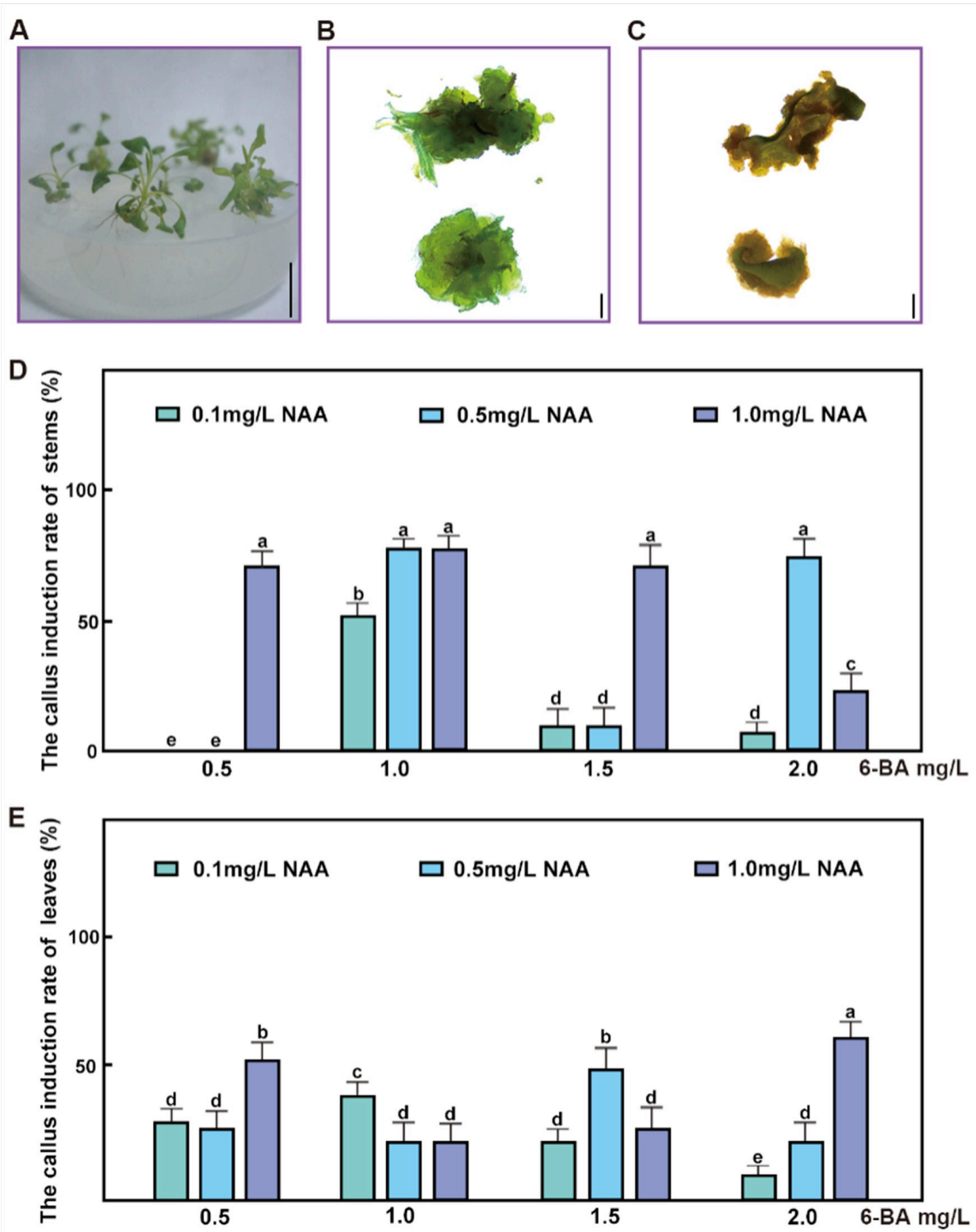


Figure 2

The callus induction of *S. angustata*

(A) Aseptic seedlings grown for 60 days, scale bar = 1 cm; (B) Callus induced from stem segments, scale bar = 1 cm; (C) Callus induced from leaf explants, scale bar = 1 mm; (D) The induction rates of stem segments under various concentrations of NAA and 6-BA; (E) The induction rates of leaves under diverse concentrations of NAA and 6-BA, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$.

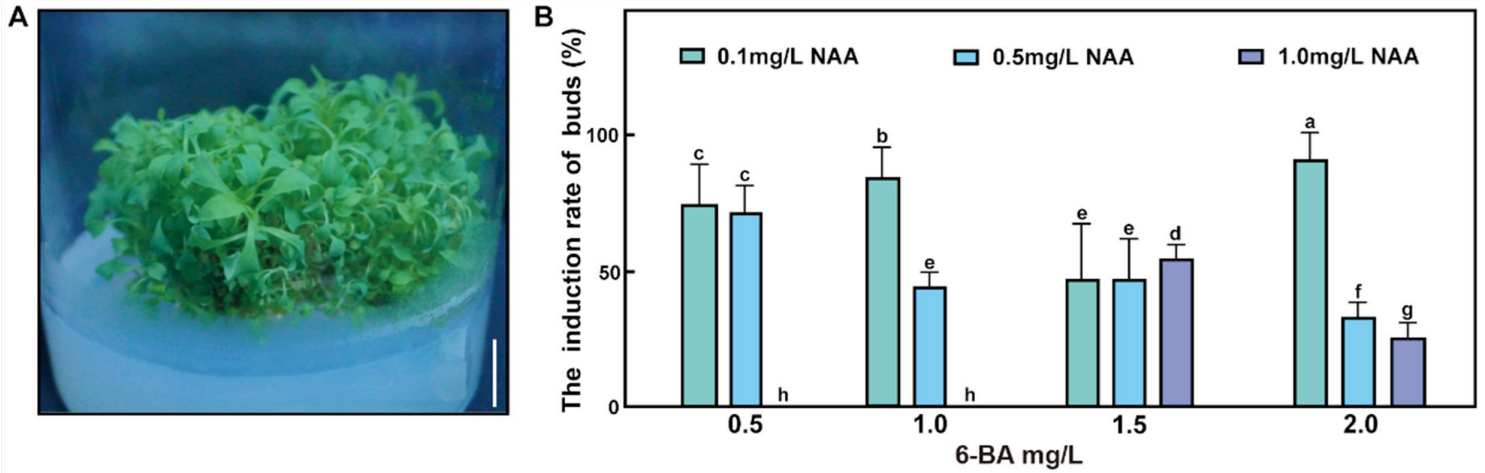


Figure 3

Effects of 6-BA and NAA on the induction rate of adventitious buds of *S. angustata*

(A) Clustered adventitious buds induced from callus, scale bar = 1 cm; (B) Effects of different concentrations of 6-BA and NAA on the adventitious bud induction rate of *S. angustata*, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$.

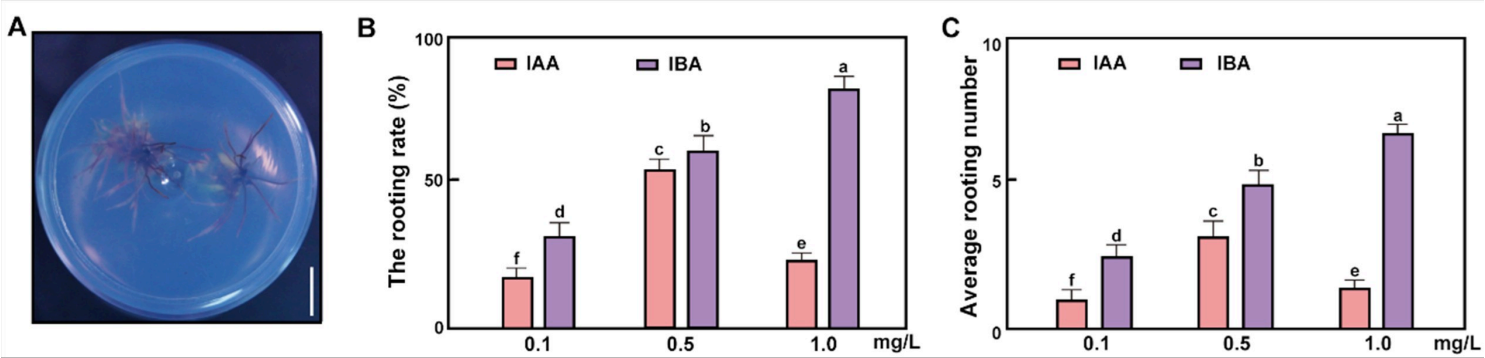


Figure 4

Effects of different concentrations of IBA and IAA on adventitious root formation of *S. angustata*

(A) Adventitious root formation of adventitious buds, scale bar = 1 cm; (B) The rooting rate after adventitious root induction from adventitious buds with different concentrations of IBA and IAA; (C) The root length after adventitious root induction from adventitious buds with different concentrations of IBA and IAA, values in the same column followed by different lowercase superscript letters indicate significant differences, $P < 0.05$.

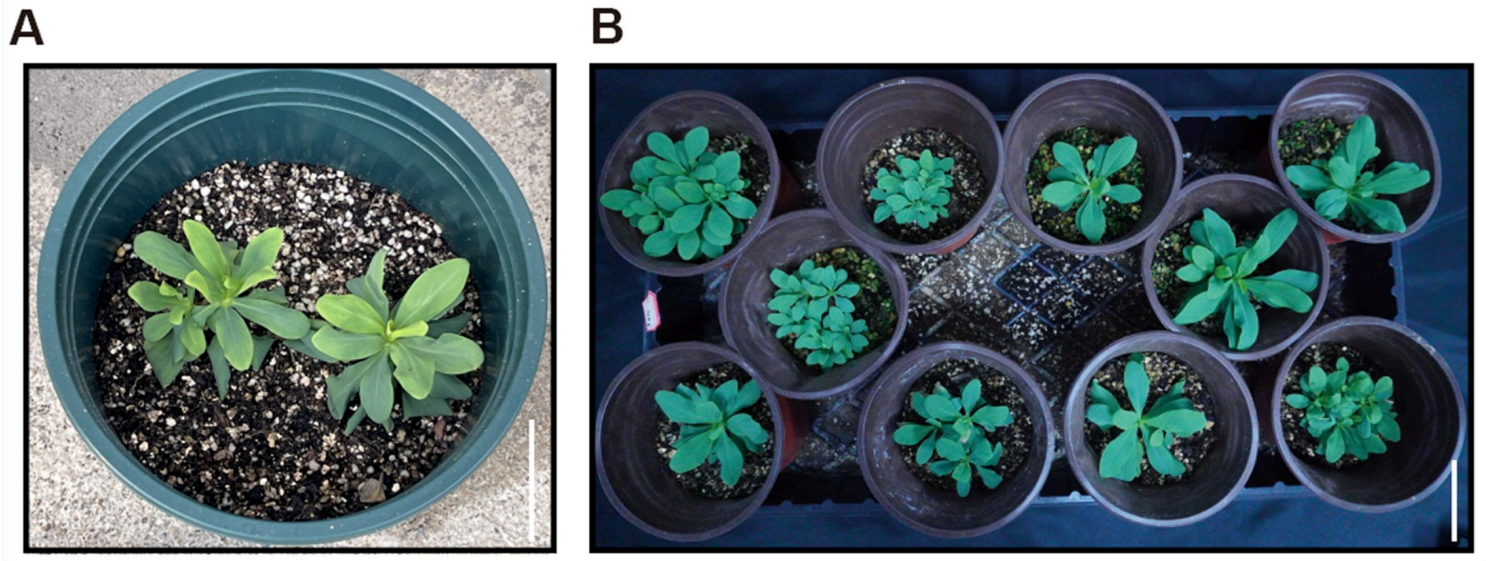


Figure 5

The habituation of *S. angustata*

(A) *S. angustata* that reproduces by seeds, scale bar = 5 cm; (B) Tissue-cultured seedlings 60 days after transplantation, scale bar = 5 cm.