

Cultivation of *Taraxum officinale* using a temporary immersion bioreactor system (TIBS)

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Abstract

Taraxacum officinale F. H. Wigg. (*T. officinale*), a perennial herb of the genus *Taraxacum* in the family Asteraceae, is widely recognized for its dual edible and medicinal properties. It has been traditionally used to treat various inflammatory conditions and exhibits significant anti-inflammatory activities. In this study, the tissue culture and rapid propagation of *T. officinale* were carried out using the temporary immersion bioreactor system (TIBS). By optimizing the immersion frequency, inoculum density and sucrose concentration, an efficient reactor-based culture system was established. The optimal conditions were determined to be an immersion frequency of 5 min/6 h, an inoculum density of 60 plantlets·L⁻¹, and the sucrose concentration of 20 g·L⁻¹. Compared with the traditional solid-state culture technique, reactor-based culture demonstrated significant advantages in plant growth morphology, proliferation rate and taraxasterol content. This research serves as a foundational framework for the mass production of taraxasterol.

Introduction

Taraxacum officinale F. H. Wigg. (*T. officinale*), a perennial herb of the genus *Taraxacum* within the Asteraceae family, is a traditional Chinese herbal medicine with dual purposes of medicine and food (Kirschner and Štěpánek, 2011). As extraction and purification techniques for the effective components of *T. officinale* continue to advance, along with growing molecular-level research, its value in pharmaceutical, food, and other applications has gained increasing recognition and attracted considerable scientific interest (Cacak-Pietrzak et al., 2021; Vural, 2024).

The bioactive compounds extracted from *T. officinale* include triterpenes, flavonoids, phytosterols, various phenolic acids, polysaccharides, coumarins and natural pigments, with 66 types of trace elements detected (Cortés et al., 2014; Ivanov et al., 2018). Taraxasterol, a pentacyclic triterpenoid derived from *T. officinale*, exhibits various pharmacological activities, including anti-inflammatory, antioxidant, and antitumor properties (Mingarro et al., 2015). Taraxasterol dose-dependently inhibited the production of inflammatory factors induced by lipopolysaccharide (LPS) in RAW 264.7 murine macrophage cells (Zhang et al., 2012). Studies have shown that taraxasterol can inhibit the production of matrix metalloproteinase-13 (MMP-13) and cyclooxygenase-2 (COX-2) induced by interleukin-1 β (IL-1 β) in human chondrocytes, and alleviate the pathological changes of articular cartilage tissue in rats with osteoarthritis. Consequently, taraxasterol demonstrates a certain protective effect against osteoarthritis (Piao et al., 2015).

The temporary immersion bioreactor system (TIBS) is a plant tissue culture system developed in recent years, which combines the advantages of maximum gas exchange in solid culture and efficient nutrient utilization in liquid culture (Mirzabe et al., 2022). It features short culture cycles, high proliferation rates, a high degree of automation, and high-throughput capacity (Nongdam et al., 2023; Orozco-Ortiz et al., 2022). In this study, *T. officinale* taken from the Jiangsu region was used as the experimental material, the temporary immersion culture research on its plantlets was conducted, and a novel production

system for *T. officinale* plantlets was established. This work lays a research foundation for the artificial production of anti-inflammatory medicinal active compounds from *T. officinale*.

Materials and methods

Materials

T. officinale plantlets were employed as experimental materials (Fig. 1B). These seedlings were obtained by cultivating sterilized seeds from wild plants in the Jiangsu region on a solid medium for 30 days. The used solid medium was based on the basal Murashige and Skoog (MS) medium, supplemented with sucrose to a concentration of $30\text{ g}\cdot\text{L}^{-1}$, agar as a supporting agent to a concentration of $7.2\text{ g}\cdot\text{L}^{-1}$, and the hormones 6-BA and GA_3 to concentrations of $0.2\text{ mg}\cdot\text{L}^{-1}$ and $1.0\text{ mg}\cdot\text{L}^{-1}$, respectively. Additionally, the pH of the medium was adjusted to 6.0.

The TIBS apparatus was acquired from Nanjing Bofang Biotechnology Co., LTD (Fig. 1A and C). It operated based on the principle of liquid temporary immersion culture, where the liquid medium periodically contacted plantlets to supply essential nutrients for their development. This equipment was composed of upper and lower structures, with the upper part being the tissue culture seedling growth chamber and the lower part being the liquid culture medium storage chamber. Air pressure was adopted as the driving force for the flow of the culture medium, and the flow frequency was programmed and controlled.

The container used for solid culture was a 200 mL glass bottle specially designed for plant tissue culture, which was purchased from Jinan Prant Biotechnology Co., LTD.

(Fig. 1)

TIBS culture of *T. officinale*

A certain number of *T. officinale* plantlets were inoculated into 100 mL of sterile liquid medium for acclimation culture over 3–5 days. After confirming the absence of contamination, the seedlings along with the liquid medium were transferred into a sterile TIBS vessel, followed by the addition of 900 mL of fresh liquid medium to complete the inoculation process. The inoculated vessel was then connected to the power and control system of the TIBS, and culture was initiated after setting the immersion frequency. The liquid medium was prepared by adding sucrose, hormones Naphthaleneacetic acid (NAA) and 6-Benzylaminopurine (6-BA) to concentrations of $30\text{ g}\cdot\text{L}^{-1}$, $0.1\text{ mg}\cdot\text{L}^{-1}$ and $0.5\text{ mg}\cdot\text{L}^{-1}$, respectively, to the basic MS medium, with the pH adjusted to 6.2. The culture environment was characterized by a light intensity of 1,800–2,000 lx, a light cycle of $14\text{ h}\cdot\text{d}^{-1}$, and a temperature of $(25 \pm 1)^\circ\text{C}$. The cultivation period was set to be 60 days.

Solid culture of *T. officinale*

During the solid culture, 200 mL tissue culture flasks were used as experimental containers. Solid culture medium was prepared and sterilized, with 50 mL dispensed into each tissue culture flask. Three plantlets were inoculated in each flask. The solid medium is to add sucrose to the basic MS medium to a concentration of $30 \text{ g}\cdot\text{L}^{-1}$, add the support AGAR to a concentration of $7.2\text{g}\cdot\text{L}^{-1}$, add the hormones α -naphthaleneacetic acid (NAA) and 6-BA to concentrations of $0.1 \text{ mg}\cdot\text{L}^{-1}$ and $0.5 \text{ mg}\cdot\text{L}^{-1}$, and adjust the pH to 6.2. The solid medium was prepared using the basal MS medium supplemented with sucrose to a concentration of $30 \text{ g}\cdot\text{L}^{-1}$, agar as a solidifying agent to $7.2 \text{ g}\cdot\text{L}^{-1}$, and the hormones NAA and 6-BA to concentrations of $0.1 \text{ mg}\cdot\text{L}^{-1}$ and $0.5 \text{ mg}\cdot\text{L}^{-1}$, respectively. The pH was adjusted to 6.2. The cultural environment was the same as that of the TIBS culture.

Optimization of cultivation conditions

The single-factor analysis method was adopted to optimize the immersion frequency, inoculum density and sucrose concentration, with four levels set for each factor.

Optimization of immersion frequency

Immersion frequency is one of the important parameters of TIBS and directly affects plant growth. The fixed inoculum density was $60 \text{ plants}\cdot\text{L}^{-1}$ and the sucrose concentration was $30 \text{ g}\cdot\text{L}^{-1}$. The immersion time was set at 5 minutes, with intervals of 1, 3, 6, and 9 hours. Each experimental group was named A1, A2, A3, and A4, respectively.

Optimization of inoculum density

The inoculum density in the reactor tank determines the utilization rate of the tank space. The sucrose concentration was fixed at $30 \text{ g}\cdot\text{L}^{-1}$ and the immersion frequency was set as 5 min/6 h. The inoculum density for each vessel was set to 20, 40, 60, and 80 explants per liter of culture medium. The corresponding experimental groups were designated as B1, B2, B3, and B4, respectively.

Optimization of sucrose concentration

Sugar serves as the carbon source for plant growth, and the concentration of sucrose in the medium directly affects the plant proliferation and growth. The fixed inoculum density was set as 60 plants per L^{-1} and the immersion frequency was 5 min/6 h. The sucrose concentration in the medium was set at 10, 20, 30, and $40 \text{ g}\cdot\text{L}^{-1}$, and each experimental group was named C1, C2, C3, and C4, respectively.

Detection of growth status and proliferation rate

After 60 days of cultivation, the plantlets of *T. officinale* were harvested. The following parameters were measured: root number, root length (cm), plant height (cm), leaf number, and leaf length-to-width ratio. The proliferation rate was calculated by the ratio of the number of plantlets per vessel to the number of inoculated explants per vessel.

Determination of taraxasterol content in plantlets

Firstly, the standard curve of taraxasterol was drawn. The taraxasterol standard solutions at varying concentrations were prepared and then analyzed by High Performance Liquid Chromatography (HPLC). A standard curve of taraxasterol concentration versus peak area was subsequently plotted based on the measured data.

Secondly, taraxasterol was extracted and its contents were determined. The plantlets of *T. officinale* from different culture systems were thoroughly washed, dried at low temperature, and ground into powder. Subsequently, 1.0 g of the powder was accurately weighed and first extracted with methanol through soaking. The solvent was then evaporated to obtain a taraxacum extract. This extract was subsequently dissolved in water and subjected to chloroform extraction. The crude extract was subjected to column chromatography to isolate single compounds. The obtained compounds were then analyzed by HPLC, and their contents were quantified based on the standard curve. HPLC conditions were as follows: The chromatographic column was a C18 column, (250 mm × 4.6 mm, 5 µm); The mobile phase was 80% acetonitrile (A) – 20% water (B); The flow rate was 1.0 mol/min; The detection wavelength was 210 nm; The column temperature was 30°C; The injection volume was 10 µL.

Results

The influence of immersion frequency on the growth status of plantlets

The morphology of plantlets showed significant differences with different immersion frequencies. Longer intervals promoted superior stem growth, whereas shorter intervals favored root elongation. As shown in Table 1, after 5 min of the immersion time, the plantlets in experimental groups A3 and A4 with longer intervals exhibited increased plant height, a greater number of leaves, and reduced length-to-width ratios of leaves (i.e., wider leaves). In groups A1 and A2 with shorter intervals, the plantlets showed shorter plants, fewer leaves, longer roots, but a reduced number of roots, and larger length-to-width ratios of the leaf (i.e., slender leaves). Generally, broader leaves result in a greater leaf surface area, which enhances the capacity for nutrient absorption. Therefore, *T. officinale* plantlets are considered healthier when they exhibit shorter leaf length and reduced leaf width. Based on the above analysis and the data statistics in Table 1, the optimal immersion frequency for *T. officinale* during the TIBS culture is 5 min/6 h.

Table 1

Effects of different culture conditions on the morphological characteristics of *T. officinale* plantlets

Treatment methods	Group	Number of roots	Root length/cm	Plant height/cm	Number of leaves	Leaf length-to-width ratio
Immersion frequency	A1	1.90 ± 0.70d	4.86 ± 0.84b	3.95 ± 0.53d	3.30 ± 0.90d	8.71 ± 0.62a
	A2	3.90 ± 1.14c	4.53 ± 0.63bc	5.17 ± 0.65c	4.20 ± 0.98c	6.97 ± 0.71b
	A3	5.50 ± 1.12a	5.12 ± 0.54a	6.58 ± 0.42a	5.50 ± 1.02a	5.00 ± 0.42d
	A4	4.90 ± 0.70b	3.72 ± 0.55c	5.47 ± 0.43b	5.10 ± 1.14b	5.51 ± 0.68c
Inoculum density	B1	6.30 ± 0.82a	6.58 ± 0.54a	6.32 ± 0.55b	5.20 ± 0.96ab	6.78 ± 0.62a
	B2	5.80 ± 0.54b	6.34 ± 0.69ab	6.68 ± 0.87a	5.10 ± 1.05bc	6.61 ± 0.58c
	B3	5.50 ± 0.76bc	5.12 ± 0.51b	6.58 ± 0.34a	5.50 ± 0.96a	5.00 ± 0.47d
	B4	3.80 ± 0.68c	3.62 ± 0.48c	4.33 ± 0.45c	3.80 ± 0.82c	7.74 ± 0.66b
Sucrose concentration	C1	4.30 ± 0.92b	4.18 ± 0.63b	3.86 ± 0.46b	3.40 ± 0.10c	5.77 ± 0.58b
	C2	5.80 ± 0.76a	5.32 ± 0.25a	6.78 ± 0.58a	6.10 ± 0.87a	5.14 ± 0.49c
	C3	5.50 ± 1.03ab	5.12 ± 0.72ab	6.58 ± 0.54ab	5.50 ± 0.88b	5.00 ± 0.41c
	C4	3.10 ± 1.05c	2.58 ± 0.33c	3.46 ± 0.67bc	2.50 ± 0.68d	7.24 ± 0.69a

Note: Data are presented as means ± standard error (SE). Within each column, values followed by different letters indicated significant differences ($p < 0.05$), as determined by DMRT.

(Table 1)

The influence of inoculum density on the growth status of plantlets

The inoculum density has a significant impact on the growth status of plantlets. Based on the data statistics and analysis presented in Table 1, the optimal growth state of the *T. officinale* plantlets was observed in group B3. Specifically, when the inoculum density was 60 plants/L of culture medium, the

seedlings exhibited greater plant height, a higher number of leaves, a reduced leaf length-to-width ratio, as well as favorable root development in both quantity and length. Although seedlings also perform well at lower inoculum densities (groups B1 and B2), the culture space is underutilized, leading to surplus nutrients and wasted resources. Conversely, an excessively high inoculum density (group B4) results in overcrowding of plantlets, which in turn produces weak growth and elongated, thin leaves.

The influence of sucrose concentration on the growth status of plantlets

TIBS culture operates as a semi-open system, allowing efficient gas exchange between the internal environment and the external atmosphere during cultivation. This facilitates an adequate supply of CO_2 for plantlets, thereby decreasing their reliance on sucrose as an external carbon source in the liquid medium. As shown in Table 1, the growth state of plantlets is the best when the sucrose concentration is $20 \text{ g}\cdot\text{L}^{-1}$ (group C2), while the sucrose concentration generally used in solid culture is $30 \text{ g}\cdot\text{L}^{-1}$. Therefore, TIBS culture can save some sucrose sources. As illustrated in Table 1, when the sucrose concentration is lower ($10 \text{ g}\cdot\text{L}^{-1}$ in group C1), the growth state of seedlings is significantly better than that at the high sucrose concentration ($40 \text{ g}\cdot\text{L}^{-1}$ in group C4). This is because the excessively high sucrose concentration increases the osmotic pressure of the culture medium, seriously affecting the absorption of nutrients by plantlets. Additionally, it also indicates that TIBS has the potential for low-sucrose or even sucrose-free culture.

Effects of different culture conditions on proliferation

The proliferation rate serves as a key indicator of cultivation capability. The proliferation rates of *T. officinale* plantlets were determined after 60 days of culture under different immersion frequencies, inoculum densities and sucrose concentrations, as shown in Fig. 2. Regarding immersion frequency, the proliferation rate obtained by group A3 (5 min/6 h) is the highest, followed by A4 (5 min/9 h), A2 (5 min/3 h) and A1 (5 min/1 h), with statistically significant differences among all groups. In terms of inoculum density, the proliferation rates of group B1 ($20 \text{ plantlets}\cdot\text{L}^{-1}$) and B3 ($60 \text{ plantlets}\cdot\text{L}^{-1}$) are the highest, and there is no significant difference between the two. The proliferation rates of B2 ($40 \text{ plants}\cdot\text{L}^{-1}$) and B4 ($80 \text{ plants}\cdot\text{L}^{-1}$) ranked third and fourth. With respect to sucrose concentration, proliferation rates of different groups can be ordered as C2 ($20 \text{ g}\cdot\text{L}^{-1}$) > C3 ($30 \text{ g}\cdot\text{L}^{-1}$) > C4 ($40 \text{ g}\cdot\text{L}^{-1}$) > C1 ($10 \text{ g}\cdot\text{L}^{-1}$), and significant differences were found across all treatment groups.

(Fig. 2)

The influence of different culture conditions on the taraxasterol content in plantlets

Taraxasterol is an important active ingredient in *T. officinale*, and its content directly affects the quality of the medicinal material. The plantlets of *T. officinale* were cultivated using TIBS. Figure 3 shows the detection results of taraxasterol content in these seedlings under different culture conditions. In terms of

immersion frequency, when the immersion time was 5 min, the taraxasterol content first increased and then decreased with the increase of the interval time. The taraxasterol content was the highest for seedlings in group A3 (with an interval of 6 h), and there were significant differences among the experimental groups. In terms of inoculum density, the taraxasterol content exhibited a similar pattern to that observed in the immersion frequency experiment, initially increasing and subsequently decreasing. The highest taraxasterol content was observed in group B3 (60 plantlets·L⁻¹), where statistically significant differences were observed across all treatment groups. The taraxasterol content showed significant differences within seedlings with different sucrose concentrations, and the highest content was observed in group C3 (at the sucrose concentration of 20 g·L⁻¹). Based on the above analysis, TIBS culture of *T. officinale* plantlets can achieve the highest taraxasterol content in groups A3, B3 and C2. This culture condition can be further applied to the artificial production of taraxasterol.

(Fig. 3)

Comparison on the growth status of plantlets in different culture methods

Traditional plant tissue cultivation relies on solid media with agar-based support. This technique uses relatively sealed small containers where effective gas exchange during the cultivation process is constrained, consequently affecting the growth of plantlets. Under the same culture environment, the growth morphology of *T. officinale* plantlets obtained from solid culture and TIBS culture was compared. Table 2 reveals the comparison results. The average number of roots in TIBS culture was 5.80, which was higher than that (3.10) in solid culture. The average height of plants and the number of leaves in TIBS culture were 6.78cm and 6.10 pieces, which were much higher than those in solid culture. There was no significant difference in the aspect ratio of leaves between the two cultivation methods. Figure 4 shows the comparison results of proliferation rates and the taraxasterol content in plantlets under different culture methods. The proliferation rate of TIBS culture reached 10.63, which was 2.81 times that of the solid culture. The content of taraxasterol in plantlets under TIBS culture was 1.04%, which was 3.51 times that of solid culture.

Table 2 Comparison of different cultivation methods on <i>T. officinale</i> plantlets					
Treatment method	Number of roots	Root length/cm	Plant height/cm	Number of leaves	Leaf length-to-width ratio
TIBS culture	5.80 ± 0.76a	5.32 ± 0.25b	6.78 ± 0.58a	6.10 ± 0.87a	5.14 ± 0.49a
Semi-solid culture	3.0 ± 0.65b	8.89 ± 0.33a	4.52 ± 0.44b	3.3 ± 0.71b	5.05 ± 0.25a

Note: Data are presented as means ± standard error (SE). Within each column, values followed by different letters indicated significant differences (p < 0.05), as determined by DMRT.

(Table 2)

(Fig. 4)

Discussion

The research on the TIBS culture of *T. officinale* (belonging to the field of plant tissue culture) is of great significance. Firstly, clonal lines of *T. officinale* can serve as a platform for fundamental research to investigate the mechanisms of apomixis. Secondly, the *T. officinale* has a high regenerative potential and can be used to study the mechanisms of organogenesis and somatic cell clonal variation during tissue culture. From the perspective of application, the *T. officinale* is an important medicinal plant (Kania-Dobrowolska and Baraniak, 2022; Li et al., 2022), containing significant active substances such as taraxasterols, taraxacum polysaccharides, and chlorogenic acid (Enteshari Najafabadi et al., 2023; Ivanov et al., 2018). It has broad-spectrum anti-inflammatory, antioxidant, anti-tumor, and diabetic treatment drug activities (Juee and Naqishbandi, 2020; Perumal et al., 2022), and has great potential in the medical application field. Furthermore, the establishment of a high-throughput TIBS cultivation system for *T. officinale* lays the foundation for the artificial production of these medicinal active substances.

The temporary immersion bioreactor system for plants is a semi-automated cultivation method developed on the basis of traditional solid and liquid plant tissue culture techniques, achieving mechanized operation and programmed control. (Mirzabe et al., 2022). By utilizing liquid medium culture, this method enables larger cultivation vessels and efficient nutrient utilization (Rico et al., 2022). The culture medium is pneumatically driven through sterile air compression, achieving effective gas exchange between the interior and exterior of the vessels (Ramírez-Mosqueda and Iglesias-Andreu, 2016). Thus, the culture cycle is shortened, plantlets grow more robustly, production efficiency is enhanced, and costs are reduced. Furthermore, through the expansion of vessel capacity and their series-parallel configuration, high-throughput cultivation is realized.

The key cultivation parameters for the temporary immersion bioreactor system are immersion frequency, inoculum density, and sucrose concentration (Georgiev et al., 2014). Among them, the immersion frequency determines the adequacy of nutrient uptake and determines whether vitrification occurs in plantlets. Relevant studies indicate that a higher immersion frequency (≥ 1 h/6 h) promotes the formation of tuberous roots and tubers, whereas lower immersion durations (≤ 1 min/12 h) enhance the formation of somatic cells and embryos (Sandal et al., 2001; Zhang et al., 2019). The immersion frequency between these ranges is optimal for seedling growth. In this study, the optimal immersion frequency for *T. officinale* cultivation was identified as 5 min/6 h, which is consistent with this pattern. The inoculum density determines the space utilization rate of the culture vessel and the growth status of the plantlets. When the inoculum density is excessively low, the available space in the vessel remains underutilized after the plantlets reach maturity, leading to resource wastage. If the inoculum density is excessively high, the plantlets will become overcrowded and unable to develop properly, as reported in the cultivation of *Bletilla striata* seedlings (Zhang et al., 2018). In this study, the *T. officinale* plantlets were used as explants, and the most suitable inoculum density was selected as 60 plantlets·L⁻¹. An excessively high or low inoculum density is unfavorable for seedling growth. The sucrose concentration

in the culture medium determines whether plantlets can grow healthily. Generally, if the sucrose concentration is too low, the plantlets will be weak and prone to browning. If the sucrose concentration is too high, the plantlets will grow excessively and aggravate vitrification (Etienne and Berthouly, 2002). The optimal sucrose concentration in the culture medium selected in this study is $20 \text{ g}\cdot\text{L}^{-1}$. At this concentration, the *T. officinale* seedlings grow healthily. Through the optimization of cultivation conditions, TIBS methods have been successfully applied in the cultivation of various plants. However, due to variations in physiological characteristics and environmental requirements among different plants, specific cultivation parameters must be individually adjusted and refined for each species.

Conclusion

In this study, the important parameters of the TIBS cultivation for *T. officinale* plantlets were optimized, and an efficient reactor-based culture system for *T. officinale* was established. This work provides a high-efficiency and low-cost method for the production of high-quality *T. officinale* plantlets and the artificial production of medicinal active substances.

Declarations

Authors Contribution BZ and FP conceived and designed the project. LS, YH and YX performed the experiments. ZS and FP analyzed the data. LS and YH wrote the first draft. All authors critically reviewed and commented on the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability All data generated during this experiment are included in this article.

Ethical approval Not applicable.

Competing interests The authors have no relevant financial or nonfinancial interests to disclose.

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Figures

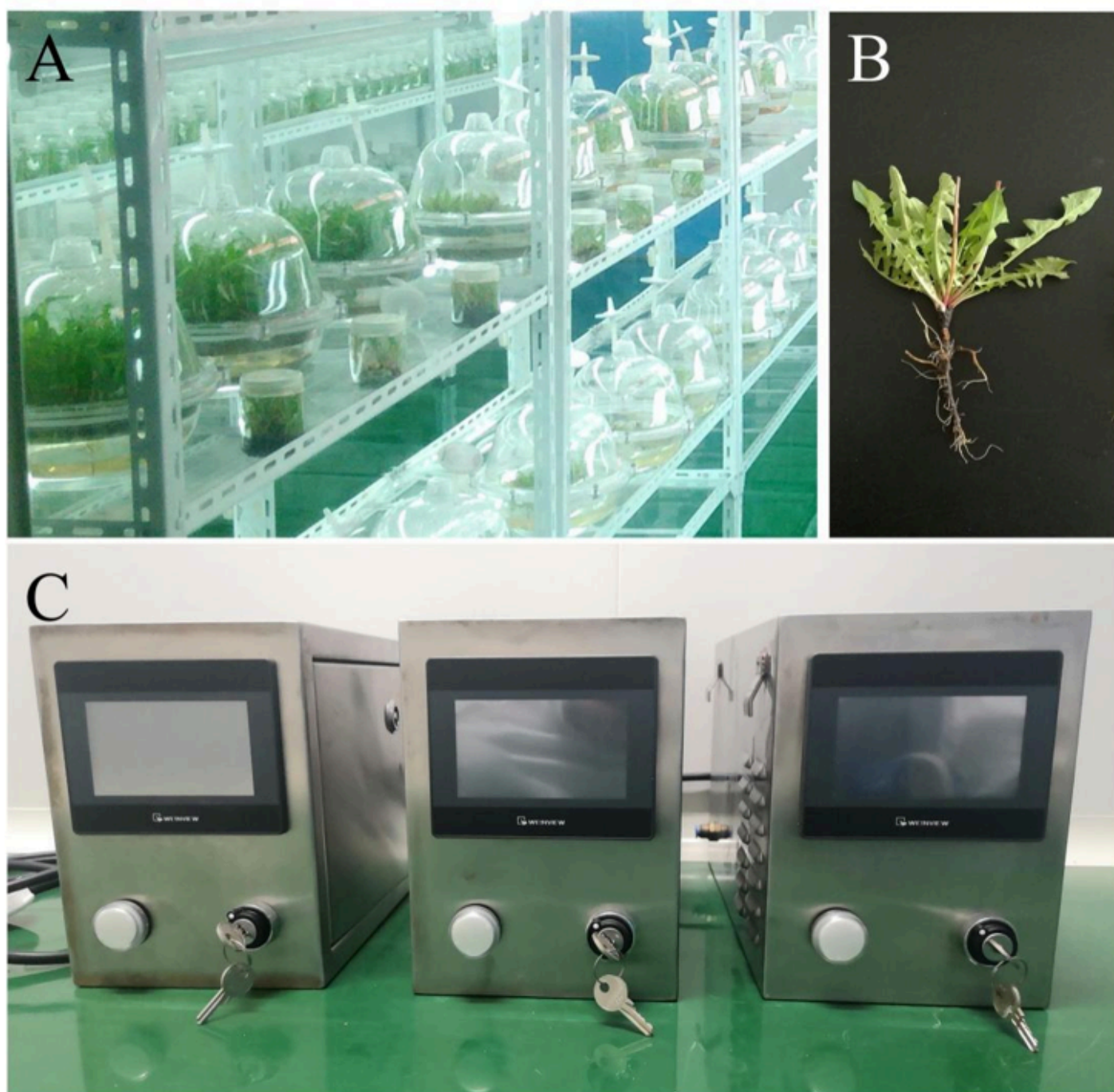


Figure 1

T. officinale plantlets and TIBS system: (A) TIBS culture of *T. officinale* plantlets; (B) *T. officinale* plantlets; (C) Control and power system of TIBS.

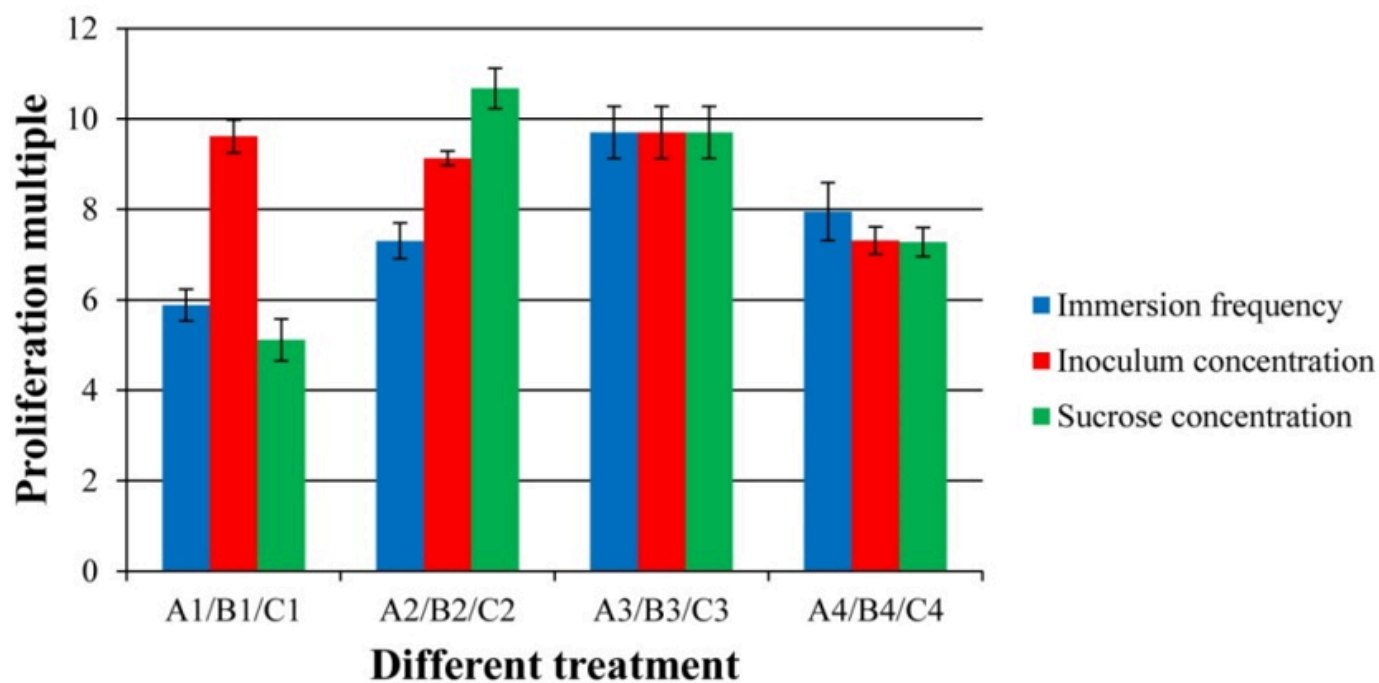


Figure 2

Effects of different culture conditions on the proliferation rate of *T. officinale* plantlets: (A) immersion frequency; (B) inoculum density; (C) sucrose concentration

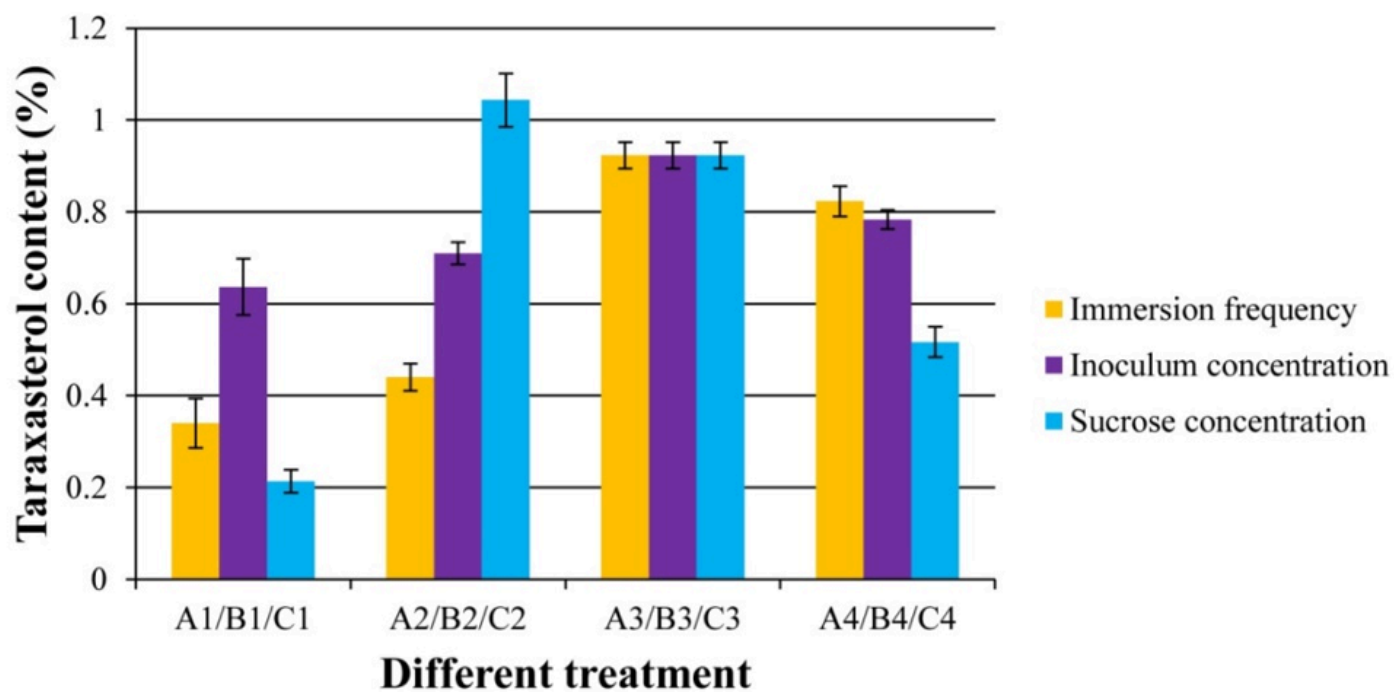


Figure 3

The effects of different culture conditions on the taraxasterol content of *T. officinale* plantlets: (A) immersion frequency; (B) inoculum density; (C) sucrose concentration

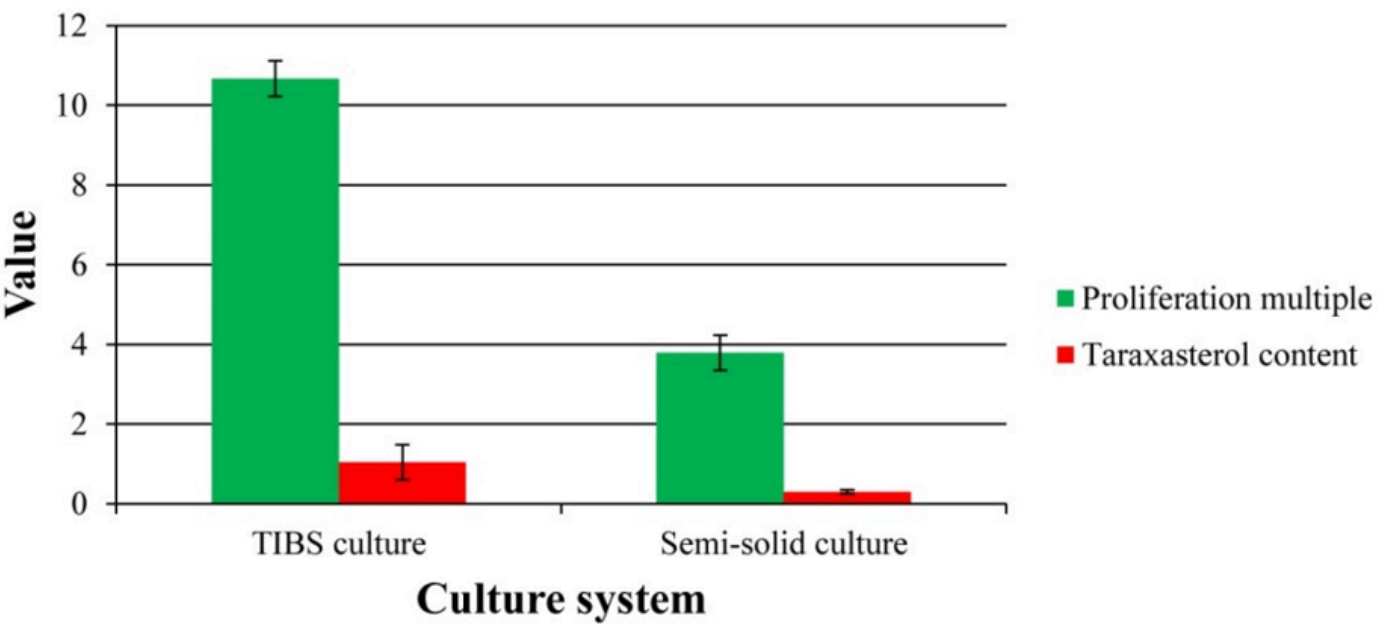


Figure 4

The proliferation rates of *T. officinale* plantlets with taraxasterol contents in different culture methods