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Optimizing planting depth and propagule characteristics for efficient cultivation of *Pinellia ternata*

Binbin Liao[1,3], Mingxing Wang[1,3], Ming Luo[1], Rong Xu[1], Jiawei Xu[1], Lu Wei[1], Chun Gui[1], Mi Lei[1], Yuhuan Miao[1]✉ & Dahui Liu[1,2]✉

***Pinellia ternata*, a common medicinal plant in East Asia, holds significant economic and therapeutic values. However, the market industrialization of the *P. ternata* is retarded due to the lack of a further understanding of its cultivation patterns. Here, we report an efficient cultivation model for *P. ternata*. This study featured a design with four planting depths (5 cm, 10 cm, 15 cm, and 20 cm) and five types of propagation materials, forming 20 distinct experimental groups. Each group was replicated three times. This study thoroughly analyzed the specific impacts of two types and five different sizes of propagules, as well as four different planting depths, on the propagation coefficient, agronomic traits, yield, and quality of *P. ternata*. (1) Tubers outperformed bulbils in propagation coefficient, agronomic traits, yield, and quality, with larger propagules showing better performance than smaller ones. (2) Small-diameter propagules (≤ 1.6 cm) achieved the best propagation coefficient, yield, and quality at a planting depth of 5 cm. (3) Large-diameter propagules (1.6–2.0 cm) showed maximum yield and quality component accumulation at 10 cm. (4) Correlation analysis indicated propagation coefficient, yield, and quality were negatively correlated with planting depth but positively correlated with propagule size. In conclusion, this study provides important theoretical support for the cultivation model of *P. ternata* and is helpful to guide its industrial production.**

Keywords Pinellia ternata, Cultivation model, Planting depth, Propagule type, Propagule size, Yield

Pinellia ternata (Thunb.) Ten. ex Breitenb., a perennial herbaceous plant of the Araceae family and the Areae tribe[1,2], is primarily distributed in East Asian countries within the North Temperate Zone, such as China, Japan, and Korea. It is also found in Germany, the Netherlands, and the eastern United States, with the most extensive distribution in central and southeastern China (https://www.gbif.org/zh/species/8678824)[3]. *P. ternata* has been a commonly used medicinal plant in East Asia, with a history of medicinal use spanning thousands of years and significant medical value[4]. In addition, *P. ternata* is included in the pharmacopeias of China and Japan, with its rhizome being the medicinal part, known for the effects of " removing dampness to reduce phlegm, downbearing counterflow to stop hiccup, and dissolving lumps and resolving masses"[5,6]. Modern pharmacological studies have shown that it possesses anti-tumor, antibacterial, anti-inflammatory, and anti-epileptic pharmacological effects, with nucleosides and organic acids being its important active ingredients[7,8]. In recent years, *P. ternata* has also been discovered to be an important plant for treating and preventing COVID-19, further expanding its range of applications[7]. Investigations show that the global demand for *P. ternata* raw materials reached 7,000 tons, with a market size of 1 billion yuan, ranking it as the 7th among the top 10 Chinese medicinal materials exported[9]. Based on its unique medical characteristics and promising market prospects, *P. ternata* has become an important industrial crop in Southeast Asian countries, possessing immense economic value and social benefits, making significant contributions to global healthcare and livelihoods.

Currently, wild resources of *P. ternata* remain one of the primary sources for commercial products[10]. However, the overharvesting of wild resources, urbanization, and the misuse of herbicides have led to a steady decline in wild *P. ternata* populations[11]. In response, many regions have begun cultivating *P. ternata* artificially to protect wild resources, increase production, and meet the robust international market demand. Despite these

[1]College of Pharmacy, Hubei University of Traditional Chinese Medicine, Wuhan 430065, China. [2]Hubei Shizhen Laboratory, Wuhan 430065, China. [3]Binbin Liao and Mingxing Wang contributed equally to this work. ✉email: miaoyh@hbtcm.edu.cn; liudahui@hbucm.edu.cn

efforts, the relatively recent history of artificial cultivation has resulted in limited experience and rudimentary management practices. Current cultivation practices for *P. ternata* often involve basic agricultural techniques that lack standardization, leading to inconsistent yields and quality. For instance, typical practices include simple planting methods without optimal consideration of propagule type and size, and planting depth, which significantly affect the crop's performance. There is a scarcity of research on cultivation models, and the lack of a standardized approach has made it challenging to maintain consistent yields and quality of *P. ternata*. As the cornerstone of the *P. ternata* industry, the cultivation challenges have emerged as the primary hindrance to its growth.

P. ternata exhibits both sexual and asexual reproduction, with asexual reproduction being predominant due to the challenges associated with sexual reproduction. Sexual reproduction in *P. ternata* relies on the development of spathes, which only form on larger propagules (> 1.5 cm) and face difficulties in fertilization and seed formation[12]. Previous studies by our research group have revealed that the type, size, and planting depth of the propagule affect the coefficient of propagation (emergences of seedlings) and yield of *P. ternata*. Moreover, studies have indicated that planting depth and propagule size substantially impact the yield and quality of root crops, making appropriate planting depths and suitable propagule sizes crucial for crop emergence, growth, and organ development[13–15]. Currently, there is a limited amount of research on the effects of tuber size and planting depth on the emergence rate and yield of *P. ternata*[16,17]. However, these studies primarily utilized yield or agronomic traits as criteria for selecting suitable planting depths or tuber sizes without assessing the bioactive components of *P. ternata*, such as nucleosides and organic acids. More importantly, bulbils, which serve as the primary propagules, are often neglected in research, severely limiting their development and application. In this study, we hypothesize that there is an optimal combination of propagule type, size, and planting depth that will not only enhance the emergence rate and yield of *P.ternata* but also improve the content of its bioactive components.

To evaluate this hypothesis rigorously, four research questions were framed: (1) Do different propagule types differentially modulate seedling emergence, agronomic performance, yield, and secondary-metabolite profiles in *P. ternata* (2) What propagule size maximizes tuber biomass while concurrently optimizing nucleoside and organic-acid concentrations (3) How does planting depth affect propagation coefficient, yield, and quality attributes (4) Which specific combination of propagule type, size, and planting depth provides a synergistic enhancement of emergence, productivity, and bioactive-metabolite accumulation?

Given this, the present study utilized the bulbils and tubers of *P. ternata* as materials for a one-year planting experiment involving multiple samples, various planting factors, and multiple replications. The primary objective was to elucidate the effects of different planting depths as well as the type and sizes of the propagules on the seedling emergence (propagation coefficient), agronomic traits, yield, content of nucleoside components, content of organic acid components, and the content of extractives in *P. ternata*. The aim was to develop an efficient cultivation mode for *P. ternata*, which is of considerable importance for the sustainable development of the *P. ternata* industry.

Materials and methods

Plant material

Propagules, including tubers and bulbils of *P. ternata*, were purchased from the producing area in Qianjiang (30º26'1"N, 112º47'23"E). It was identified by Professor Dahui Liu from Hubei University of Chinese Medicine as the plant *P. ternata* from the Araceae family; the varieties are shown in Figure 1. The tubers are categorized into three size grades: T1 (2.0–1.6 cm), T2 (1.4–1.2 cm), and T3 (1.0–0.8 cm). The bulbils are divided into two size grades: B1 (1.0–0.8 cm) and B2 (0.8 –0.6 cm).

Planting

The experiment was conducted from December 2021 to December 2022 at the Medicinal Plant Garden of Hubei University of Chinese Medicine in Wuhan, Hubei Province, located at latitude 30°27′6″N and longitude 114°15′51″E. The area falls within a subtropical monsoon humid climate zone, with an elevation of 20 m, an average annual temperature of 19.5 °C, an average annual sunshine duration of 1741.4 h, and an average annual precipitation of 1260 mm. The terrain is flat and well-arranged, suitable for the growth of *P. ternata*. A randomized complete block design was employed: the experiment was set up with four planting depths of 5 cm, 10 cm, 15 cm, and 20 cm, and utilized five types of propagation materials as detailed in "Plant material", creating 20 different experimental groups with three replicates each (Fig. S1). In the experimental pots, 36 experimental materials were planted, three rows were set up, each row spaced 9 cm apart, with 12 propagules per row spaced 4.5 cm apart. During the growth of *P. ternata*, pest, disease, and weed control were managed in accordance with conventional field practices.

Determination of agronomic traits and extract content

Seven indicators, including agronomic traits and extractable content, were measured using the following methods: (1) Seedling emergence: After the emergence of *P. ternata* seedlings is completed in late April and August 2022, count the number of seedlings in the experimental plots (Seedling emergence = Number of seedling petioles/number of propagules); (2) Leaf length: After *P. ternata* stops growing in June 2022, measure the length of the middle split leaf blades; (3) Leaf width: After *P. ternata* stops growing in June 2022, measure the width of the middle split leaf blades; (4) Plant height: After *P. ternata* stops growing in late June 2022, measure the height of the plant; (5) Petiole diameter: After *P. ternata* stops growing, in June 2022 measure the diameter of the petiole at the middle part; (6) Yield (biomass): After *P. ternata* falls over (late November 2022), collect the tubers and bulbils, cleaning them of soil, and then weighing them. (7) Extract content: The measurement was conducted using the cold maceration method as recorded in General Rules 2201 of the 2020 edition of the

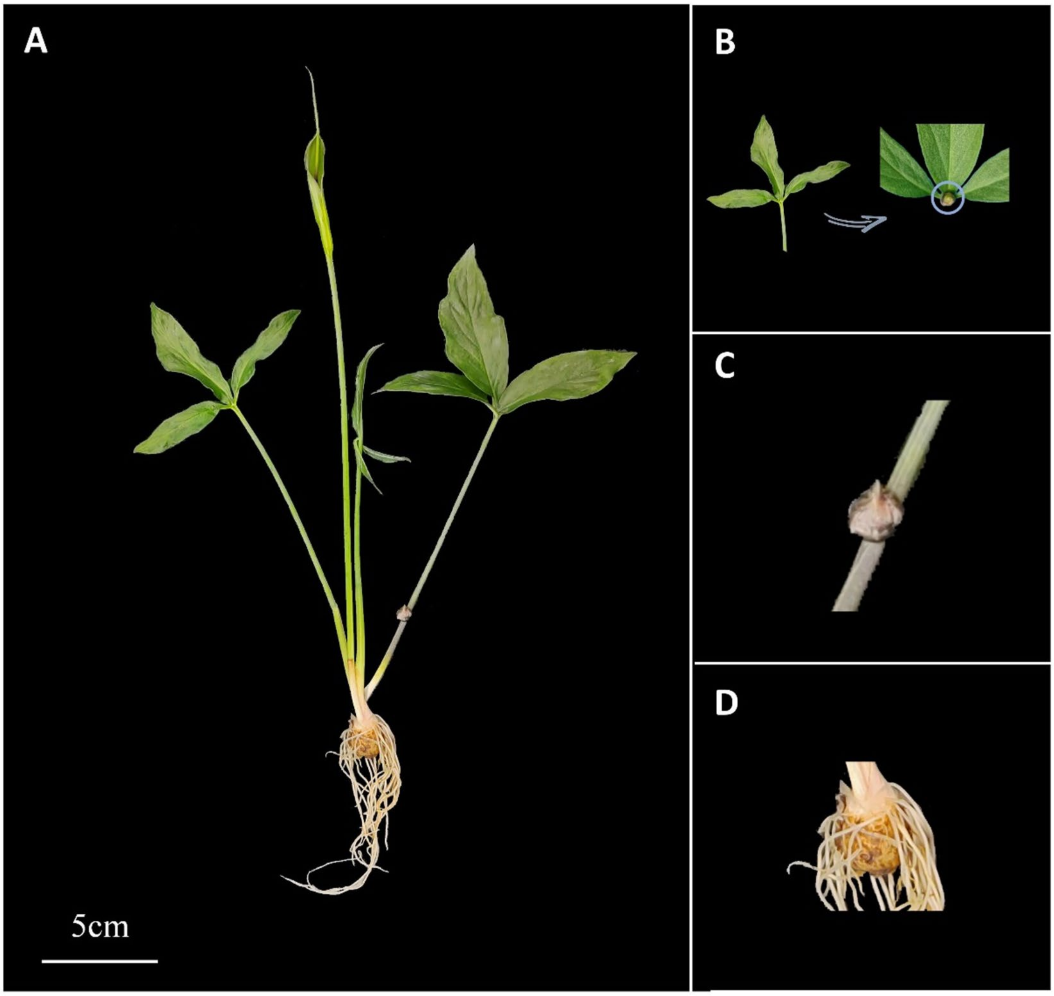

Fig. 1. *P. ternata* plants and propagation materials. **A** *P. ternata* plants; **B** leaf bulbil; **C** stem bulbil; **D** tuber.

Chinese Pharmacopoeia[5]. The measurements of the aforementioned agronomic traits were all taken using a vernier caliper.

Determination of nucleoside

Based on previously published research[18], we identified six nucleoside components of tubers in *P. ternata*, and their contents were determined using high-performance liquid chromatography (HPLC). The HPLC analysis was conducted on an Agilent 1260 HPLC system. Separation was achieved on a column (Agilent XDB-C18, 4.6 mm × 250 mm, 5 µm) at a constant flow rate of 1 mL/min at 25 °C. To determine the contents of uracil, hypoxanthine, uridine, inosine, guanosine, and adenosine, the mobile phase consisted of water (A) and acetonitrile (B) with a gradient elution program as follows: 0–10 min, 0.5–2% B; 10–20 min, 2–3.5% B; 20–30 min, 3.5% to 10% acetonitrile; 30–50 min, 10% to 28% acetonitrile; 50–55 min, 28% to 30% acetonitrile; 55–60 min, 30% to 0.5% acetonitrile. The injection volume was 10 µL, and the monitored wavelength was 260 nm. The results of the standard measurements are shown in Fig. S2. Regression equations were established with the concentration of each component on the x-axis and the peak area integral values (w) on the y-axis, as shown in Table S1.

Determination of organic acid

Based on previously published research by Wei et al. (2024)[19], we identified five organic acids, including malic acid, succinic acid, citric acid, cis-aconitic acid, and fumaric acid, from the tubers of *P. ternata*, and their contents were determined using HPLC-MS. The analysis was conducted on an Agilent 1260 HPLC system coupled with an Agilent Ultivo triple quadrupole mass spectrometer. Separation was performed on a column (Agilent ZORBAX Eclipse Plus C18, 100 mm × 2.1 mm, 1.8 µm) at a constant flow rate of 1 mL/min at 25 °C. For separating these chemical components, the injection volume was two µL, and the mobile phase consisted of 1% formic acid in water (A) and acetonitrile (B) with 5% B isocratic elution. The electrospray ionization source was operated in negative ion mode, and multiple reaction monitoring mode was used to detect the five organic acids simultaneously. Additionally, the drying gas temperature was set to 325 °C with a flow rate of 8 L/min; the sheath gas temperature was 350 °C with a flow rate of 11 L/min; the nozzle voltage was 45 psi, and the spray voltage was 3839 V. Table S2 presents the mass spectrometry parameters and regression equations.

Data analysis

Statistical analyses were performed using GraphPad Prism version 10.4.0. The data were analyzed to assess the effects of propagule type, size, and planting depth on the propagation coefficient, agronomic traits, yield, and quality of Pinellia ternata. The following statistical methods were employed: (1) Correlation Analysis: Import the mean values of propagule size, planting depth, agronomic traits, yield, extractable content, nucleoside components, and organic acid components into Statistic 26, and perform Pearson correlation analysis with a two-tailed significance test. The resulting data is then imported into Chiplot (https://www.chiplot.online/) to create a correlation heatmap. In this analysis, propagule size and planting depth were considered as independent variables, while agronomic traits, yield, and bioactive component content were considered as dependent variables. (2) Multifactorial ANOVA: A multifactorial analysis of variance (ANOVA) was conducted to evaluate the significant variations in planting depth and propagule size. The ANOVA included planting depth and propagule size as independent factors and the various agronomic traits and yields as dependent variables.

Results

The emergence rate of *P. ternata* seedlings under different planting depths and propagule sizes

The plants, bulbils, and tubers of *P. ternata* are shown in Fig. 1. Bulbils and tubers of different sizes were selected as propagules for planting experiments in this research. Two major peaks of seedling emergence were observed within the cultivation cycle, occurring in April and August, while two main peaks of seedling lodging were noted in June and November. Detailed records of the emergence status of *P. ternata* were kept monthly throughout the entire cultivation period, with the data presented in Fig. 2. This study found that the planting depth had no significant impact on the emergence rate of the tubers in the T1 group; however, it was negatively correlated with the number of seedlings in the other planting groups. Further analysis indicated that under all examined

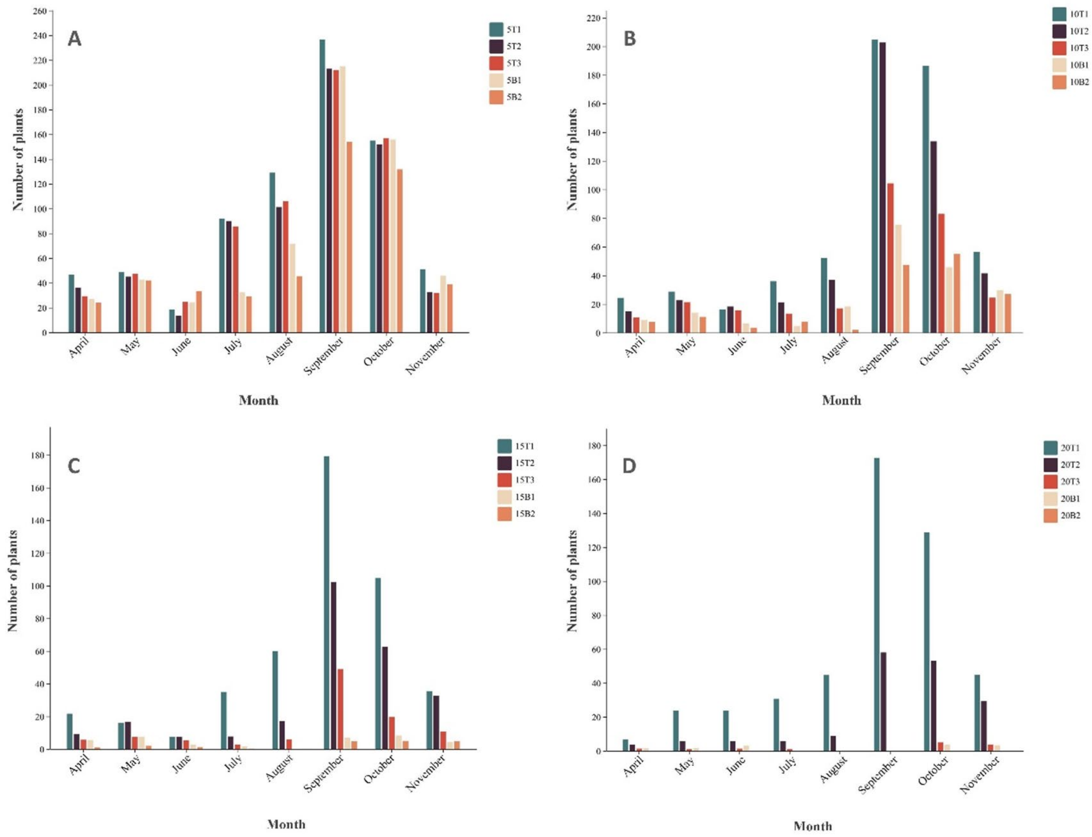

Fig. 2. Seedling emergence of *P. ternata* at different sowing depths for various particle sizes. **A** The number of seedlings of different particle sizes of *P. ternata* at a depth of 5 cm; **B** The number of seedlings of different particle sizes of *P. ternata* at a depth of 10 cm; **C** The number of seedlings of different particle sizes of *P. ternata* at a depth of 15 cm; **D** Number of *P. ternata* seedlings of different particle sizes at a depth of 20 cm. T1: tubers size in 2.0–1.6 cm; T2: tubers size in 1.4–1.2 cm; T3: tubers size in 1.0–0.8 cm; B1: bulbils size in 1.0–0.8 cm; B2 bulbils size in 0.8 –0.6 cm.

planting depths, the T1 group had the highest number of seedlings, followed by the T2, T3, and B1 groups, while the B2 group had the fewest seedlings.

At a planting depth of 5 cm, all sizes of propagules were able to germinate successfully. Among them, the T1 group showed the highest emergence of seedlings, while the B2 group showed the lowest. The emergence of seedlings for the T2, T3, and B1 propagules was relatively balanced, indicating that under shallow planting conditions, there is no significant difference in the emergence of seedling numbers between tubers and bulbils of the same size. It is worth noting that larger tubers germinated earlier and also lodged earlier. Moreover, with increasing planting depth, the emergence of seedlings for all tubers showed a declining trend, demonstrating a negative correlation between the emergence of seedlings and planting depth, with smaller-sized tubers being more affected by planting depth and having fewer emergences of seedlings. At a planting depth of 10 cm, the larger-sized T1 and T2 group tubers were less affected by planting depth, showing their stronger tolerance to deep planting, while the emergence of seedlings of other sizes of propagules was more significantly impacted. When the planting depth reached 15–20 cm, the emergence of seedlings for B1 and B2 grade bulbils was very low, or they failed to germinate at all, indicating that this depth is unsuitable for planting smaller-sized *P. ternata*. However, at a planting depth of 15 cm, the T3 group tubers still had a certain number of emergences of seedlings, indicating that tubers have a stronger tolerance to deep planting than bulbils under the same size conditions. After the first emergence, the emergence of seedlings in the second emergence period was significantly higher than in the first, and September was the period with the highest emergence of seedlings for *P. ternata*. Overall, the results from the entire cultivation cycle indicate that the emergence of seedlings of *P. ternata* is positively correlated with the size of the tuber and negatively correlated with planting depth. Tubers, as propagation materials, have a more substantial tolerance to deep planting than bulbils.

The main phenotypic traits of *P. ternata* under different planting depths and propagule sizes

During the peak seedling period in June 2022, groups with fewer than five seedlings were excluded (15B2, 20T3, 20B1, 20B2), and a statistical analysis of the main phenotypic traits of the remaining groups of *P. ternata* was conducted. The data on the main phenotypic traits of *P. ternata* under different planting depths and propagule sizes were analyzed using GraphPad Prism version 10.4.0. As illustrated in Fig. 3, the results showed that the agronomic traits of *P. ternata* have a significant positive correlation with the size of the propagules, and no significant correlation was found with planting depth. Under the same depth conditions, propagules with larger diameters significantly outperformed those with smaller diameters in leaf length, plant height, and petiole diameter. However, the T1 group of tubers reached the maximum leaf width at the same planting depth, while other groups did not show significant differences in leaf width. The impact of different planting depths on the four agronomic traits varied; plant height significantly decreased with increasing planting depth, while leaf length and width did not significantly change with depth. The petiole diameter showed little variation across different depths but tended to increase with greater planting depth.

The yield and extract content of *P. ternata* under different planting depths and propagule sizes

Among the sample groups with yields exceeding 10 g (as seen in Fig. 4A), after excluding the 15B1, 15B2, 20T3, 20B1, and 20B2 samples, an analysis was conducted on the yield and extractable content of propagules at different depths. The study results indicated that yield is negatively correlated with planting depth and positively correlated with the size of the propagules. The yield and extractable content were measured at the end of the growth period in late November 2022, after the plants had lodged and the tubers were harvested.

As shown in Fig. 4B, Group T1 demonstrated a significant advantage over other groups under the 5 cm planting depth condition. The yield of the tuber groups T2 and T3 was higher than that of the bulbil groups B1 and B2. Although there were differences in particle size between groups T2 and T3 at planting, their final yields were similar, as were the yields of groups B1 and B2. Moreover, the smallest B2 grade bulbils could yield a high output, significantly contributing to the overall yield increase. Under the 10 cm planting depth condition, the size of the propagules had a more pronounced effect on yield, with the larger propagules showing a more pronounced tolerance to deep planting. The yield of group 10T1 was higher than that of 5T1, indicating that the largest T1-grade tubers are more suitable for planting at a depth of 10 cm. For other groups, as the diameter of the propagules decreased, the yield gradually decreased, and all were lower than the yields under the 5 cm planting depth. Under the 15 cm and 20 cm planting depth conditions, the yield of T1 changed little but was lower than the yields under the 10 cm and 5 cm planting depths. For other groups, as the particle size decreased, the yield dropped sharply, indicating that depths of 15 cm and 20 cm are not suitable for cultivating *P. ternata*.

In this study, the content of extractable matter in all experimental groups of *P. ternata* exceeded the minimum standard of 7.5% as stipulated by the Chinese Pharmacopoeia, with an average extractable content of 16.04%, as shown in Fig. 4C. The results indicated that when the planting depth was 5 cm, the extractable content of *P. ternata* reached its highest value of 17.49%, whereas at a planting depth of 10 cm, the extractable content dropped to its lowest at 15.09%. Furthermore, at planting depths of 15 cm and 20 cm, the average extractable content of *P. ternata* was 15.21% and 16.03%, respectively. Further analysis revealed that both the B1 and B2 groups exhibited higher levels of extractable content regardless of whether they were planted at depths of 5-10 cm.

The contents of nucleoside components of *P. ternata* under different planting depths and propagule sizes

This study conducted a quantitative analysis of nucleoside components in *P. ternata*, with detailed data in Table 1. The analysis revealed significant differences in the content of the six measured nucleosides. The nucleoside components were measured at the end of the growth period in late November 2022, after the plants had lodged and the tubers were harvested.

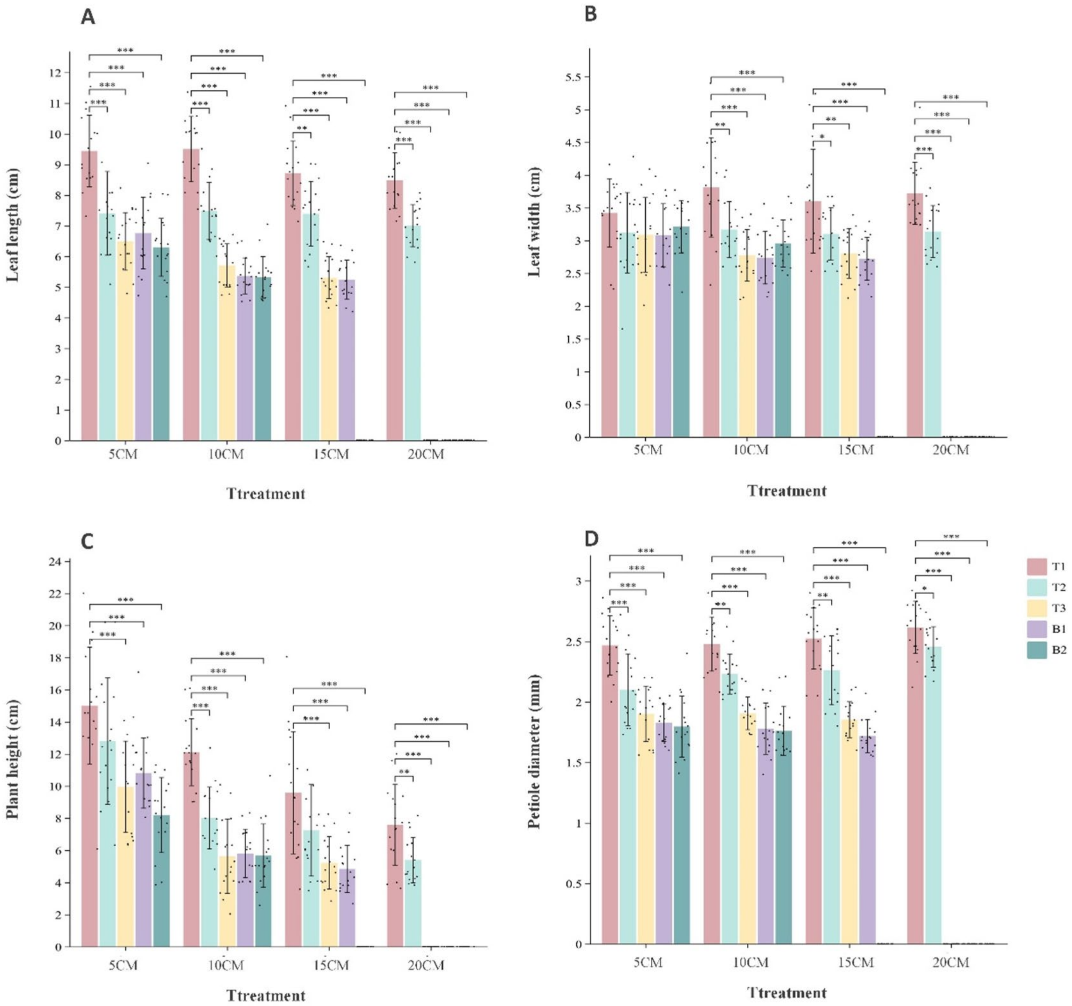

Fig. 3. Main agronomic traits of *P. ternata* at different planting depths and particle size levels. **A** Leaf length; **B** leaf width; **C** plant height; **D** petioles diameter.

Specifically, the contents of uridine, guanosine, and adenosine were relatively high, with average contents of 154.88 μg/g, 143.91 μg/g, and 95.84 μg/g, respectively. In contrast, uracil had the lowest content, with an average of only 8.50 μg/g, while hypoxanthine and inosine had contents of 13.36 μg/g and 20.58 μg/g, respectively. Among all the sample groups, the 10T1 group had the highest total nucleoside content, reaching 675.17 μg/g, followed by the 10B2 group and the 5B2 group, with total contents of 537.88 μg/g and 512.07 μg/g, respectively. Conversely, the 20T2 group had the lowest total nucleoside content, with a sum of the six nucleosides at 285.13 μg/g.

The study clarified the impact of planting depth on the accumulation of nucleoside components in *P. ternata*. The sample group with a planting depth of 10 cm had the highest accumulation of nucleoside components, reaching 518.09 μg/g, followed by the sample group with a planting depth of 5 cm, with an average total nucleoside content of 471.11 μg/g. Subsequently, the sample groups with planting depths of 15 cm and 20 cm had total contents of 337.00 μg/g and 299.60 μg/g, respectively. The results indicated that most nucleoside components, including uracil, uridine, guanosine, and adenosine, exhibited a trend of increasing and then decreasing with the increase in planting depth, peaking at a planting depth of 10 cm. However, the content of hypoxanthine gradually decreased with increasing planting depth. These findings reveal that the accumulation of nucleoside components in *P. ternata* is significantly affected by planting depth and particle size, with the total content of the six nucleosides reaching its maximum value at a planting depth of 10 cm.

The contents of organic acid of *P. ternata* under different planting depths and propagule sizes

The content of organic acids in *P. ternata* varies significantly, with citric acid being the highest, followed by succinic acid and malic acid, while fumaric acid and cis-aconitic acid have the lowest content, as shown in Table 2. The organic acid components were measured at the end of the growth period in late November 2022, after the plants had lodged and the tubers were harvested.

The experimental results indicate that the group with a planting depth of 10 cm has the highest total accumulation of the five organic acids, with an average total content of 20.61 μg/g, followed by the group with

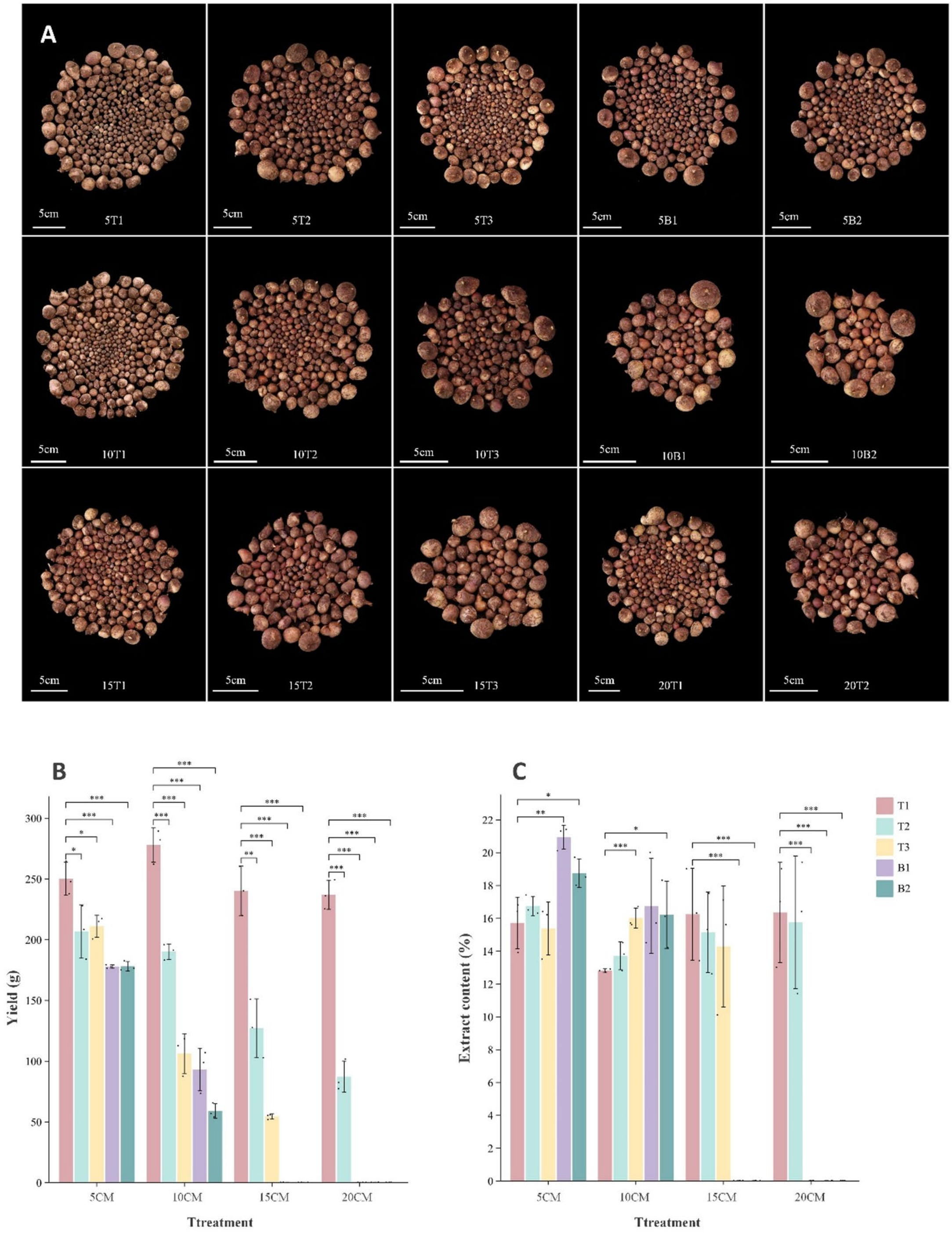

Fig. 4. **A** Yield of *P. ternata* at different planting depths and particle size grades; **B** statistics of yield; **C** statistics of leachate content.

a planting depth of 5 cm, with an average total content of 19.62 μg/g. The groups with planting depths of 15 cm and 20 cm have the lowest contents, with average totals of 14.81 μg/g and 17.82 μg/g, respectively.

In the groups where tubers were used as propagules, the propagules of size T2 (1.2–1.4 cm) showed a specific advantage at different depths. Furthermore, at a planting depth of 5 cm, the average total content of the T2 group was 20.16 μg/g, which was higher than that of the T1 and T3 groups. The same situation occurred in groups with planting depths of 10 cm and 15 cm. It was found that under the same depth, the content of the five organic acids changed with the size of the particle diameter, increasing first, peaking at T2, and then decreasing.

Group	Uracil	Hypoxanthine	Uridine	Inosine	Guanosine	Adenosine	Total
5T1	10.02 ± 1.05	20.63 ± 1.19	133.71 ± 2.97	59.28 ± 3.50	174.02 ± 11.52	89.95 ± 1.19	487.61
5T2	3.47 ± 0.52	14.83 ± 0.43	140.96 ± 14.15	10.32 ± 0.98	120.84 ± 2.67	90.89 ± 8.61	381.31
5T3	3.17 ± 0.27	14.45 ± 0.71	165.99 ± 5.22	9.33 ± 0.54	169.65 ± 7.52	123.50 ± 10.72	486.09
5B1	3.71 ± 0.56	11.32 ± 0.68	187.86 ± 5.28	9.32 ± 3.76	149.57 ± 15.92	126.70 ± 8.98	488.48
5B2	8.57 ± 1.89	26.98 ± 2.10	189.34 ± 14.48	18.41 ± 0.77	139.76 ± 6.82	129.01 ± 3.34	512.07
10T1	16.17 ± 0.48	20.05 ± 0.72	248.78 ± 10.89	34.52 ± 3.15	196.02 ± 3.77	159.63 ± 10.48	675.17
10T2	5.94 ± 0.18	13.99 ± 1.00	159.63 ± 12.46	27.33 ± 3.24	143.96 ± 10.12	111.16 ± 10.14	462.01
10T3	9.29 ± 1.32	9.63 ± 2.17	170.17 ± 15.71	6.38 ± 0.21	148.04 ± 12.03	97.62 ± 11.31	441.13
10B1	15.40 ± 1.97	16.14 ± 2.32	174.31 ± 7.76	23.46 ± 3.59	148.57 ± 6.63	96.38 ± 3.29	474.26
10B2	11.09 ± 1.08	13.01 ± 1.25	190.36 ± 14.37	10.29 ± 1.48	174.73 ± 14.8	138.40 ± 13.90	537.88
15T1	6.49 ± 0.28	4.96 ± 0.62	144.70 ± 14.39	13.04 ± 0.44	130.87 ± 2.71	16.36 ± 1.23	316.42
15T2	11.18 ± 2.06	8.94 ± 0.45	118.02 ± 9.25	20.03 ± 3.25	112.67 ± 6.77	96.58 ± 5.70	367.42
15T3	10.80 ± 1.58	11.92 ± 0.39	95.18 ± 67.52	37.56 ± 4.05	111.58 ± 6.47	60.14 ± 2.57	327.18
20T1	6.09 ± 0.27	5.17 ± 0.29	130.73 ± 3.11	11.41 ± 0.51	120.53 ± 5.85	40.10 ± 47.30	314.03
20T2	6.10 ± 0.43	8.44 ± 1.07	73.50 ± 52.98	18.07 ± 1.03	117.88 ± 7.96	61.14 ± 3.84	285.13

Table 1. Content of nucleoside components in *P. ternata* at different cultivation modes.

Group	Fumaric acid	Succinic acid	Citric acid	Malic acid	cis-Aconitic acid	Total
5T1	0.37 ± 0.02	1.03 ± 0.04	13.43 ± 0.31	2.14 ± 0.10	0.39 ± 0.03	17.33
5T2	0.39 ± 0.01	1.04 ± 0.04	15.56 ± 0.23	2.85 ± 0.04	0.38 ± 0.01	20.16
5T3	0.37 ± 0.01	0.95 ± 0.03	14.51 ± 0.59	2.83 ± 0.16	0.33 ± 0.01	18.98
5B1	0.39 ± 0.01	0.75 ± 0.04	16.45 ± 0.31	3.58 ± 0.27	0.41 ± 0.01	21.53
5B2	0.49 ± 0.01	0.38 ± 0.12	16.00 ± 1.05	2.88 ± 0.06	0.33 ± 0.01	20.08
10T1	0.43 ± 0.00	0.96 ± 0.01	15.09 ± 0.20	2.96 ± 0.16	0.37 ± 0.00	19.72
10T2	0.46 ± 0.00	1.17 ± 0.05	18.81 ± 0.59	2.99 ± 0.15	0.40 ± 0.01	23.82
10T3	0.40 ± 0.02	0.99 ± 0.03	16.75 ± 0.28	2.17 ± 0.13	0.40 ± 0.01	20.66
10B1	0.48 ± 0.00	0.96 ± 0.05	15.68 ± 0.21	1.89 ± 0.16	0.37 ± 0.03	19.30
10B2	0.41 ± 0.01	0.88 ± 0.05	15.88 ± 0.20	2.16 ± 0.17	0.29 ± 0.04	19.54
15T1	0.42 ± 0.01	0.94 ± 0.03	12.10 ± 0.05	0.99 ± 0.08	0.32 ± 0.03	14.77
15T2	0.44 ± 0.01	0.95 ± 0.06	15.13 ± 0.47	1.01 ± 0.12	0.37 ± 0.01	17.87
15T3	0.30 ± 0.01	0.64 ± 0.06	10.03 ± 0.33	0.71 ± 0.07	0.14 ± 0.00	11.79
20T1	0.30 ± 0.01	1.04 ± 0.00	14.91 ± 0.54	1.47 ± 0.35	0.35 ± 0.01	18.06
20T2	0.29 ± 0.02	1.01 ± 0.01	14.73 ± 2.39	1.23 ± 0.08	0.34 ± 0.02	17.57

Table 2. Content of organic acid components in *P. ternata* at different cultivation modes.

Correlation analysis

This research unveiled the correlations among propagule size, planting depth, agronomic traits, yield, extractable content, nucleoside components, and organic acid components (Fig. 5). It was found that propagule size has a significant positive correlation with agronomic traits. However, its impact on chemical components is variable, mainly showing a significant positive correlation with succinic acid content. Planting depth affected the chemical composition of *P. ternata* significantly, with a significant negative correlation observed between planting depth and the content of fumaric acid, malic acid, hypoxanthine, guanosine, and inosine in the plant.

Furthermore, this research indicated a slight correlation between agronomic traits and chemical components, such as a negative correlation with fumaric acid, citric acid, guanine, adenosine, and extractable content and a positive correlation with succinic acid, cis-aconitic acid, and inosine, but these correlations were not significant. Notably, petiole diameter showed a negative correlation with almost all chemical components. Among organic acids, citric acid and cis-aconitic acid were positively correlated with the content of other organic acids. And fumaric and malic acid were positively correlated with the content of most alkaloids. The alkaloid chemical components did not show specific correlation information, but it is worth noting that, except for uracil, the rest of the alkaloids were significantly negatively correlated with planting depth. Extractable content showed a negative correlation with propagule size, planting depth, and agronomic traits, but the correlations were not significant. Yield showed a significant positive correlation with propagule size and all agronomic traits and a negative correlation with planting depth, but the correlation was not significant.

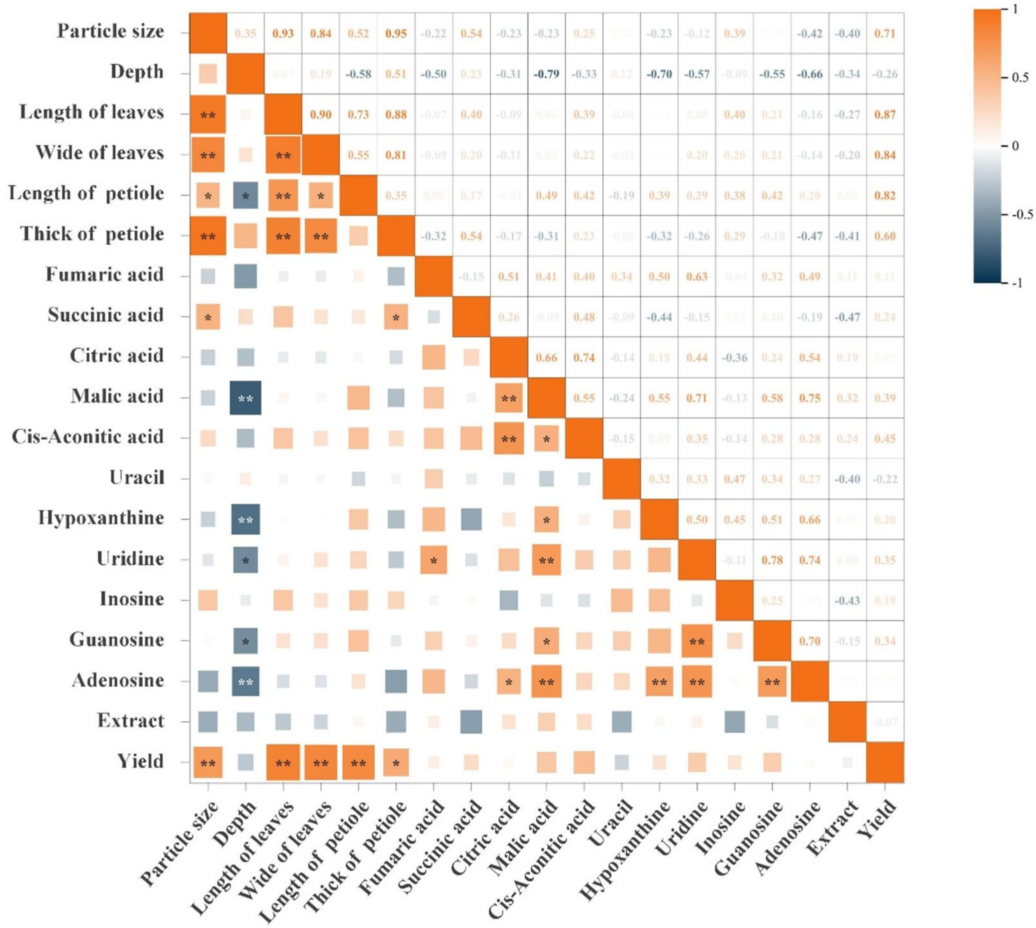

Fig. 5. The correlations among particle size, planting depth, agronomic traits, yield, extractable content, nucleoside components, and organic acid components.

Discussion

The type of propagule, its size, and planting depth can broadly influence the growth and development of various plants[20,21]. Thus, it is of significant importance for the industrial production of *P. ternata* to determine the appropriate type and size of propagules, as well as the corresponding planting depth.

The research demonstrated that different types of propagules significantly affect the yield of *P. ternata*, with tuber yields being markedly higher than those of bulbils. It's important to note that bulbils can be categorized into stem and leaf bulbils, which, as crucial elements of asexual reproduction, possess considerable potential for application. In the natural environment, once mature, bulbils naturally detach and, upon entering the soil, facilitate propagation[22]. Therefore, in the cultivation of *P. ternata*, it is recommended to select varieties that include bulbils to fully exploit the benefits of asexual reproduction, thereby enhancing the propagation efficiency and yield of *P. ternata*.

The study reveals that the type and size of propagule and planting depth can significantly influence the growth and development of various rhizomatous plants, including *P. ternata*[23]. It is crucial to select larger bulbils or tubers as propagules to enhance the propagation efficiency and yield of *P. ternata*. Research indicates that larger propagules can significantly improve the emergence of seedlings of *P. ternata*, a result that aligns with previous studies[24]. This phenomenon may be attributed to the fact that larger propagules typically contain richer nutrient reserves, providing ample energy and material foundations for initial growth and development, thereby promoting rapid sprouting and emergence[25,26]. Furthermore, larger propagules can reach their maximum biomass more quickly, and under high-density planting conditions of *P. ternata*, the reduction in aboveground biomass is lower compared to smaller propagules[27,28]. Additionally, studies have pointed out that plants derived from larger tubers have a stronger competitive ability compared to those from smaller tubers. This could be because plants from larger propagules have better adaptability and recovery capabilities under adverse conditions, thus exhibiting stronger competitiveness[29,30]. Moreover, plants from larger propagules also have more developed root systems, enhancing their ability to absorb nutrients[31].

The research findings indicate that the size of propagules has a significant positive impact on agronomic traits and yield. Specifically, larger propagules tend to show higher emergence of seedlings and tillering capabilities, and they exhibit superior characteristics in terms of agronomic traits, such as increased leaf area[32,33]. These traits contribute to enhanced photosynthetic efficiency and biomass accumulation[34], which are directly correlated with the yield of *P. ternata*.

This study reveals that *P. ternata* is best suited to shallow planting depths, a finding that aligns with the characteristic of its shallow root system[35]. As the planting depth increases, the emergence of seedlings and yield of *P. ternata* significantly decrease, consistent with previous research[17,36]. Furthermore, this study revealed that intense planting leads to uneven and delayed emergence of seedlings, similar to the findings of Xiao et al. (2000)[16]. A possible explanation is that significant changes in planting depth can markedly alter the abiotic conditions of *P. ternata* cultivation, including oxygen, temperature, moisture, and soil, all of which are not conducive to the formation of suitable conditions for the emergence of seedlings[37,38]. On the other hand, increased planting depth may also directly inhibit the growth of *P. ternata* propagules. Given the limited nutritional reserves stored in *P. ternata* propagule, excessive nutrient consumption during emergences of seedlings and emergence could lead to insufficient nutrient supply after the propagule has germinated, resulting in weak seedlings[39], which is consistent with previous research[17], which found that propagules with a diameter greater than 2 cm of *P. ternata* can tolerate deeper planting but result in poor seedling conditions. Additionally, smaller rhizomes may exhaust all stored carbohydrates before the new shoots reach the soil surface, increasing the risk of plant regeneration and thus affecting the emergence of seedlings[40]. The difficulty in the emergence of seedlings observed in this study for 15B1/B2/20T3/B1/B2 also confirms this point.

It is worth noting that a planting depth that is too shallow may cause bulbils to emerge above the ground, and after lodging, they may remain on the ground without soil cover, affecting reproduction efficiency. Therefore, to enhance reproduction efficiency and the yield of *P. ternata*, attention should be paid to its growth cycle, especially after the lodging periods (April and August), when immediate soil covering should be carried out.

One of the quality control indicators for *P. ternata* is the content of water-soluble extractives according to the regulations of the Chinese Pharmacopoeia, which may include organic acids, saponins, alkaloids, sugars, amino acids, and proteins, among other components. This study showed that as the planting depth increases, the content of extractives tends to decrease, a phenomenon also confirmed by two other indicators. Alkaloids and organic acids, as key bioactive components of *P. ternata*, show a significant negative correlation with planting depth for most of their chemical constituents. It is speculated that increased planting depth leads to reduced soil permeability, affecting the respiratory function of the crop's root system. Given the close relationship between the synthesis of nucleosides and organic acids and plant metabolic activities, insufficient soil permeability may limit these metabolic processes, thereby negatively impacting the synthesis of these components[41]. Moreover, the temperature in deeper soil layers is typically lower than in the topsoil, and temperature is a key factor affecting enzyme activity and metabolic reactions[42]. Lower soil temperatures may slow the biosynthesis rate of nucleosides and organic acids. To ensure the quality and yield of *P. ternata*, it is recommended to use shallow planting.

Bulbils, as a key organ for the propagation of *P. ternata*, play an indispensable role. Given the impact of genetic diversity on *P. ternata*, some varieties may lack bulbils or have low bulbil activity. Hence, screening for varieties with superior bulbil characteristics holds significant potential value for improving the production efficiency of *P. ternata*. Furthermore, previous studies have revealed that during the growth process of *P. ternata* seeds, the fresh weight of the plant shows an increasing and then decreasing trend, while the fresh weight and diameter of the tubers exhibit an "S" shaped change pattern over time[43]. Based on the actual observation of the growth dynamics of *P. ternata*, determining the appropriate planting and harvesting periods has become a research topic that requires in-depth exploration.

Conclusion

This study analyzed the impact of different types and sizes of *P. ternata* propagules, as well as planting depth, on the propagation coefficient, agronomic traits, yield, and quality of *P. ternata* by planting experiments, contributing to the development of efficient cultivation patterns for *P. ternata*. Tubers outperformed bulbils regarding propagation coefficient, agronomic traits, yield, and quality; larger propagules also performed better than smaller ones in the abovementioned aspects. Additionally, the study revealed the adaptive relationship between the yield and quality of *P. ternata* propagules of different particle sizes and planting depth, with the optimal planting depth for small-sized (≤ 1.6 cm) propagules being 5 cm. For larger-sized (1.6–2.0 cm) propagules, the optimal planting depth is 10 cm, at which the highest propagation coefficient, yield, and quality can be achieved. Furthermore, the propagation coefficient, yield, and quality of *P. ternata* are negatively correlated with planting depth and positively correlated with the size of the propagules. In summary, this research provides valuable insights into the scientific cultivation of *P. ternata*, aiding in its industrial development and benefiting growers, traders, and consumers alike.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files.

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References

1. Wu, Z. & Peter, H. R. *Flora of China*. Vol. 23 (Science Press Beiing & Missouri Botanical Garden Press, 2012).
2. Zhang, J. et al. Germplasm resources, genetic diversity, functional genes, genetic breeding, and prospects of *Pinellia ternata* (Thunb.) breit: a review. *Med. Plant. Biol.* **2**, 1–8 (2023).
3. *Pinellia ternata* (Thunb.) Makino in GBIF Secretariat. *GBIF Backbone Taxonomy. Checklist Dataset* (2023). https://www.gbif.org/zh/species/8678824. Accessed via GBIF.org on 29 Nov 2024.

4. Zou, T. et al. A review of the research progress on *Pinellia ternata* (Thunb.) Breit.: Botany, traditional uses, phytochemistry, pharmacology, toxicity and quality control. *Heliyon* **9**, e22153 (2023).
5. Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People's Republic of China* (China Medical Science and Technology, 2020).
6. Editorial Committee of the Japan Pharmaceutical Bureau. *The Japanese Pharmacopoeia* (Japanese Ministry of Health, Labour and Welfare, 2016).
7. Bai, J. et al. A comprehensive review on ethnopharmacological, phytochemical, pharmacological and toxicological evaluation, and quality control of *Pinellia Ternata* (Thunb.) Breit. *J. Ethnopharmacol.* **298**, 115650 (2022).
8. Chen, C. et al. *Pinellia* genus: A systematic review of active ingredients, Pharmacological effects and action mechanism, toxicological evaluation, and multi-omics application. *Gene* **870**, 147426 (2023).
9. Zhiwei, Z., Yanran, L. & Haiying, L. Study on current situation of import and export trade and industrialization development of *Pinelliae rhizoma*. *J. Chin. Med. Mater.* **45**, 1021–1028 (2022).
10. Yike, X. et al. Genetic diversity analysis and molecular id card construction of *Pinellia Ternata* based onphenotypie traits and SSR markers. *Chin. J. Exp. Tradit Med. Formulae*. **30**, 134–142 (2024).
11. Mao, R. & He, Z. *Pinellia Ternata* (Thunb.) breit: A review of its germplasm resources, genetic diversity and active components. *J. Ethnopharmacol.* **263**, 113252 (2020).
12. Dexing, G., Yunxiang, L. & Bingsheng, X. A study on the reproductive biology of *Pinellia ternata* (Thunb.) Breit. *J. Plant. Resour. Environ.* **3**, 44–48 (1994).
13. Sarwar, G. et al. Revealing the dynamics of saffron growth: optimizing corm size and planting depth for increased yield synergies. *Plos One*. **19**, e303264 (2024).
14. Singh, M. K., Kumar, S., Ram, R. & Prasad, R. Effect of size of bulb, growing conditions and depth of planting on flower and bulb production of Asiatic hybrid Lily (*Lilium* spp) cv. Brunello. *Indian J. Agr Sci* **86,** 391-393 (2016).
15. Crawford, L. E. & Williams, M. M. Planting depth and seed size affect Edamame emergence individually. *Hortscience* **54**, 92–94 (2019).
16. Jieyi, X., Tingjie, S., Zhongbao, W., Zhouqiang, H. & Shengcai, B. The impact of different planting depths on the yield of *Pinellia ternata* (brief report). *Chin. J. Chin. Mater. Med.* **25**, 49–50 (2000).
17. Wenjun, L., Xiulan, Y., Jianwen, P., Jiancang, C. & Peng, W. The influence of stem size, sowing depth, and sowing period on the emergence and seedling situation of *Pinellia ternata*. *J. Chin. Med. Mater.* **33**, 1534–1536 (2010).
18. Yinzhu, L. et al. Effects of different drying methods on drying characteristics and appearance and components of *Pinelliae rhizoma*. *Chin. Trad Herb. Drugs*. **52**, 5845–5853 (2021).
19. Wei, L. et al. Response surface modelling of six organic acids from *Pinellia ternata* (Thunb.) Breit by ultrasound-assisted extraction and its determination by high-performance liquid chromatography coupled to triple quadrupole mass spectrometry. *Chemosensors* **12**, 47 (2024).
20. Kimmelshue, C. L., Goggi, S. & Moore, K. J. Seed size, planting depth, and a perennial groundcover system effect on corn emergence and grain yield. *Agronomy-Basel* **12** (2022).
21. Mangoale, R. M. & Afolayan, A. J. Effects of rhizome length and planting depth on the emergence and growth of *Alepidea amatymbica* Eckl. & Zeyh. *Plants-Basel* **9**, 732 (2020).
22. Shu, F. et al. Bulbil initiation: a comprehensive review on resources, development, and utilisation, with emphasis on molecular mechanisms, advanced technologies, and future prospects. *Front. Plant. Sci.* **15**, 1343222 (2024).
23. Martínez-Ballesta, M. C., Alcaraz-López, C., Muries, B., Mota-Cadenas, C. & Carvajal, M. Physiological aspects of rootstock–scion interactions. *Sci. Hortic.* **127**, 112–118 (2010).
24. Hanyan, W., Leixia, C., Mengjuan, Y., Wenchuan, H. & Chengming, D. Quality evaluation of different propagation methods of *Pinellia ternate*. *Lishizhen Med. Mater. Med. Res.* **35**, 709–714 (2024).
25. Jia, Z., Yongqi, H. & Fei, W. Current advances on the molecular mechanism of seed Vigor. *J. South. Chin. Agr Univ.* **44**, 659–669 (2023).
26. Li, X., Shen, Y., Huang, Q., Fan, Z. & Huang, D. Regeneration capacity of small clonal fragments of the invasive *Mikania micrantha* H.B.K.: Effects of burial depth and stolon internode length. *Plos One*. **8**, e84657 (2013).
27. Boccaccini, A. et al. When size matters: new insights on how seed size can contribute to the early stages of plant development. *Plants-Basel* **13**, 1793 (2024).
28. Ambika, S., Manonmani, V. & Somasundaram, G. Review on effect of seed size on seedling vigour and seed yield. *Res. J. Seed Sci.* **7**, 31–38 (2014).
29. Hanász, A., Zsombik, L., Magyar-Tábori, K. & Mendler-Drienyovszki, N. Effect of drought and seed tuber size on agronomical traits of potato (*Solanum tuberosum* L.) under in vivo conditions. *Agronomy-Basel* **14**, 1131 (2024).
30. Ji, X., Tang, J. & Zhang, J. Effects of salt stress on the morphology, growth and physiological parameters of *Juglans microcarpa* L. Seedlings. *Plants-Basel* **11**, 2381 (2022).
31. Zierer, W., Rüscher, D., Sonnewald, U. & Sonnewald, S. Tuber and tuberous root development. *Annu. Rev. Plant. Biol.* **72**, 551–580 (2021).
32. Stockem, J. E., Bus, M. D., de Vries, M. E. & Struik, P. C. Opening eyes on seedling tuber quality in potato: size matters. *Potato Res.* **67**, 1691–1715 (2024).
33. Park, H. et al. Effects of seed tuber size on dormancy and growth characteristics in potato double cropping. *Hortic. Environ. Biote.* **64**, 167–178 (2023).
34. De Souza, A. P. et al. Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science* **377**, 851–854 (2022).
35. Xu, J. et al. A novel *Pinellia ternata* catalase gene *PtCAT2* regulates drought tolerance in *Arabidopsis* by modulating ROS balance. *Front. Plant. Sci.* **14**, 1206798 (2023).
36. Hailing, W., Yingjun, T., Jun, Z., Zhengkang, P. & Xudong, Z. Study on soil cover thickness for planting different stems of *Pinellia ternata*. *Bull. Agr Sci. Technol* 6, 110–113 (2023).
37. Yunqi, X., Caiyun, Z. & Xiangjian, Z. Effects of burial depth and planting density on seedling emergence and growth of *Ambrosia artemisiifolia* of different seed sizes. *Acta Ecol. Sin.* **41**, 9621–9629 (2021).
38. Rezvani, M., Nadimi, S., Zaefarian, F. & Chauhan, B. S. Environmental factors affecting seed germination and seedling emergence of three *Phalaris* species. *Crop Prot.* **148**, 105743 (2021).
39. Sapkota, T. B. et al. Crop nutrient management using nutrient expert improves yield, increases farmers' income and reduces greenhouse gas emissions. *Sci. Rep.* **11**, 1564 (2021).
40. Aguirre, M., Kiegle, E., Leo, G. & Ezquer, I. Carbohydrate reserves and seed development: an overview. *Plant. Reprod.* **31**, 263–290 (2018).
41. Duan, X. et al. Compacted soil adaptability of *Brassica napus* driven by root mechanical traits. *Soil. Tillage Res.* **233**, 105785 (2023).
42. Steinweg, J. M., Dukes, J. S. & Wallenstein, M. D. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. *Soil. Biol. Biochem.* **55**, 85–92 (2012).
43. Yunfei, L. *Research on Tuber Expansion Regular of Different Propagation Materials and Soilless Cultivation of Pinellia ternata* (Huazhong Agricultural University, 2014).

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Author contributions
MW designed the experiment. BL and Ming Luo performed the experiments. RX, JX, and Mi Lei helped in data collection. CG and LW commented the manuscript. BL and MW wrote the paper and discussed it with all authors. DL and YM supervised the study and provide funding. All the authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Y.M. or D.L.