Research Square

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In vitro germination and storage optimization of Fraxinus chinensis pollen

Hu Sun
Shenyang Agricultural University
Huiyan Guo
Shenyang Agricultural University
Xiaoyu Ji
Shenyang Agricultural University
Yueliang Wu
wuyueliang72@syau.edu.cn
Shenyang Agricultural University

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Abstract

Fraxinus chinensis is a precious broad-leaved tree species with economic, ecological and ornamental values. Cross-breeding enables the development of novel germplasms with accelerated growth rates, enhanced stress tolerance and improved ornamental performance. Accordingly, pollen viability assessment and storage research are essential for its controlled artificial hybridization. In this study, the medium for *in vitro* pollen germination of *F. chinensis* was optimized. The optimized medium consisted of 150 $g{\cdot}L^{-1}$ sucrose, 0.1 $g{\cdot}L^{-1}$ boric acid, and 0.05 $g{\cdot}L^{-1}$ calcium nitrate at pH 6.0. Based on this superior medium, pollen germination rates were examined at different temperatures, and 25℃ was identified as the most favorable temperature for *in vitro* pollen germination. Further studies revealed that thawing at 4℃ for 24 h followed by rehydration at 25℃ for 4 h significantly enhanced pollen germination, achieving a rate of 91.49%, indicating that controlled thawing and rehydration greatly improved pollen vitality. Storage experiments revealed that *F. chinensis* pollen could maintain high viability at 25℃ and 4℃ (suitable for short-term storage), whereas − 20℃ and − 80℃ were found to be effective for long-term preservation. This finding was considered to provide important support for pollination in cross-breeding of *F. chinensis*.

1. Introduction

As a deciduous tree of the genus *Fraxinus* in the Oleaceae family, *Fraxinus chinensis* has an extensive distribution across northern and southern China and parts of East Asia, and is also present in Europe and North America (Liu et al., 2025; Hou et al., 2026). It has high-quality timber with versatile applications, and its bark is used as traditional herbal medicine (Wang et al., 2024). Meanwhile, it serves as a major tree species for landscaping, ecological restoration and shelterbelt construction (Chen et al., 2020). Nevertheless, *F. chinensis* is an anemophilous tree species with abundant phenotypic variation among natural hybrids. Controlled pollination enables pyramiding elite agronomic traits and breeding superior ash cultivars. Consequently, investigations into pollen viability assessment and pollen storage protocols hold great theoretical and practical significance for interspecific hybridization and germplasm conservation of *Fraxinus* species.

Pollen germination has been shown to be affected by a multitude of factors. It has been well documented that sucrose, boric acid, Ca^{2+} concentration and medium pH predominantly regulate *in vitro* pollen viability, germination and pollen tube elongation (Li et al., 2023; Jia et al., 2022), as exemplified by *foxtail millet*, whose optimal sucrose concentration for peak pollen germination is 15% (w/v) (Sun et al., 2024). In rice, pollen germination is optimal at 20% sucrose, with lower levels reducing the germination rate and excessively high concentrations causing pollen tube rupture (Yohana et al., 2025). Furthermore, boric acid facilitates pollen water uptake and hydration, breaks pollen dormancy and initiates germination. Multiple studies have confirmed that boric acid at a concentration of 100 mg/L is optimal for pollen germination of a wide range of plant species (Weng et al., 2023; Araújo de Oliveira et al., 2021). Besides, numerous studies have demonstrated that Ca^{2+} acts as a vital regulator of pollen germination and pollen tube growth in plants (Steinhorst et al., 2013; Matsuura-Tokita et al., 2026). As reported previously, investigations into optimal *in vitro* germination media for *Ipomoea batatas* and *Carya illinoinensis* revealed maximum pollen germination at calcium nitrate concentrations of 300 mg/L and 750 mg/L, respectively (Weng et al., 2023; Wang et al., 2021). Also, a favorable pH contributes to ROS balance and Ca^{2+} uptake, whereas abnormal pH conditions suppress pollen development (Liu et al., 2020). The optimum medium pH for maximum *in vitro* pollen germination of quinoa is 5.5, whereas *Exochorda racemosa* pollen attains peak germination and pollen tube elongation at pH 6.5 (Castillo et al., 2022; Jia et al., 2022). Moreover, culture temperature is the primary factor governing pollen viability (Tushabe et al., 2025). A study displayed that the preferred temperature range for pollen germination and pollen tube growth in *Mangifera indica* is 27.9–32.1℃ (Liu et al., 2023). For *Dimocarpus longan* pollen, the maximum germination rate is observed at room temperature (23–24℃), while the lowest rate occurs at 10–20℃ (Pham et al., 2015). In addition, proper storage temperatures can effectively sustain pollen viability. Pollen of various plant species stored at 4℃, -20℃ and − 80℃ exhibits significantly higher survival rates than pollen kept at room temperature (Mehmood et al., 2025). For example, jujube pollen achieves the optimum germinability of 65% when preserved at 4℃ (Kadri et al., 2022). Apple pollen stored at -20℃ and − 80℃ exhibits germination rates 14–15 fold higher than those under room temperature storage (Ćalić et al., 2021). Pollen preservation at different temperature is applicable to short- and long-term storage (Li et al., 2025; Nagar et al., 2023). For long-term conservation, one study found that pepper pollen stored at -80℃ for one year retained over 90% pollination capacity (Du et al., 2025).

In vitro pollen germination is the most common method to test pollen viability in woody plants (Rose et al., 2023). The optimal culture system for *in vitro* pollen germination is highly species-specific. To date, systematic studies on pollen viability evaluation and *in vitro* germination techniques for *F. chinensis* remain insufficient. The optimum medium components (including concentrations of sucrose, boric acid, Ca^{2+} as well as pH value), suitable germination temperature, and storage conditions, and other related factors have not been clarified, which greatly limits the efficiency of its cross-breeding. Accordingly, this study aims to establish a standardized incubation system for pollen viability and preservation of *F. chinensis*. This study will fill the research gaps regarding pollen viability and *in vitro* germination of *F. chinensis*, providing practical guidance for artificial pollination and cross-breeding, as well as valuable insights for germplasm conservation and the selection of elite cultivars.

2. Materials and methods

2.1. Plant material and pollen harvest

Pollen grains were collected from robust 15-year-old *F. chinensis* trees located in the Botanical Garden of Shenyang Agricultural University (41°49'26.03" N, 123°33'32.93" E), which features a temperate continental monsoon climate. In late April 2025, male inflorescences with newly bloomed but indehiscent anthers were sampled at the pollen shedding stage. Mature pollen grains were stripped from stamens onto sulfuric acid paper, air-dried in a cool shaded place at room temperature for 24 h, and subsequently divided into 1.5 mL centrifuge tubes at approximately 1.0 mL pollen per tube.

2.2 Screening of pollen culture medium

Pollen viability was determined via the *in vitro* germination assay. An orthogonal array test strategy (OATS) was adopted to screen the optimal medium formulation for pollen germination, with four factors including sucrose (S), boric acid (B), calcium nitrate (C) and pH (P) set at three concentration levels (Table 1) (Zhao et al., 2021; Yohana et al., 2025). Pollen grains were evenly spread onto the medium surface, and Petri dishes were sealed and incubated at 25℃ for 48 h in a growth chamber. Four biological replicates were established for each medium treatment; for each replicate, approximately 100 pollen grains were observed per field of view. Mean pollen germination percentage was quantified after microscopic observation using a Nikon LY-WN-HPCCD30 microscope (Tokyo, Japan). A pollen grain was defined as germinated when its pollen tube length exceeded twice the diameter of the pollen grain (Pham et al., 2015). The OATS design follows the formula $L_N(V^K)$, where N refers to the number of experimental combinations, V denotes the maximum level number per factor, and K is the total number of tested factors. The orthogonal experimental design $L_9(3^4)$ consisting of nine treatment combinations (three concentration levels with four replicates) was analyzed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, Illinois, USA). Range analysis, analysis of variance (ANOVA) and multiple comparison were performed to identify the optimal culture medium for pollen germination.

Table 1
Combination of medium compositions for optimizing *F. chinensis* pollen germination by using an orthogonal assay test strategy (OATS)

Levels	Sucrose (S)($g \cdot L^{-1}$)	Boric acid (B)($g \cdot L^{-1}$)	Calcium nitrate (C)($g \cdot L^{-1}$)	pH
1	50	0.05	0.05	5
2	100	0.1	0.1	6
3	150	0.2	0.2	7

2.3 Optimization of incubation temperature and duration for pollen germination

After determining the optimal medium formulation for pollen germination, pollen grains were uniformly spread on the optimized medium in petri dishes and incubated independently under six temperature regimes (5, 10, 15, 20, 25 and 30℃) at a

constant relative humidity of 85%. All temperature treatments were conducted in programmable growth incubators. Samples were assessed for pollen germination at five incubation time points (3, 6, 12, 24 and 48h), with four biological replicates set for each treatment. Around 100 pollen grains were counted per microscopic field, and the total pollen grain count and number of germinated grains were recorded individually.

2.4 Thawing, rehydration and *in vitro* germination of cryopreserved pollen

Pollen preserved at -80℃ for 7 days was taken out and thawed in a refrigerator at 4℃, and pollen germination rates were detected after 0 h, 12 h and 24 h of thawing. Subsequently, pollen with different thawing durations was rehydrated for 0 h, 2 h and 4 h in a sealed container at 25℃ with 97% relative humidity, before being evenly spread onto germination medium. The individual and combined effects of thawing and rehydration treatments on the recovery of pollen viability were analyzed.

2.5 Storage conditions and *in vitro* germination of pollen

Freshly collected pollen of *F. chinensis* was air-dried at room temperature for 24 h, followed by measurement of initial germination percentage. The pollen was then divided into 1.5 mL centrifuge tubes and stored at four temperatures (25℃, 4℃, -20℃, -80℃). Changes in pollen viability were determined at six storage periods: 7 d, 14 d, 30 d, 60 d, 180 d and 365 d. For frozen samples, pollen was first thawed at 4℃ for 24 h and rehydrated at 254℃ for 4 h in a sealed container prior to inoculation on medium. Pollen stored at 25℃ and 4℃ was subjected to the identical rehydration treatment to unify experimental conditions. Pollen viability across different storage temperatures and durations was compared to screen the optimal pollen preservation regime.

2.6 Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, Illinois, USA). The OATS method was applied to evaluate the effects and optimum levels of medium components on pollen germination. Multiple comparisons were conducted via the Student-Newman-Keuls (S-N-K) test embedded in SPSS Statistics 17.0.

3. Results

3.1 Optimization of *in vitro* germination medium for *F. chinensis* pollen

To determine the optimal medium formulation for *in vitro* germination of *F. chinensis* pollen, four medium components each set at three concentration levels were tested (Table 1). The pollen germination performance of *F. chinensis* on different screening media is shown in Fig. 1. Panels a-i in Fig. 1 illustrate pollen germination characteristics for the nine treatment combinations of the orthogonal experiment listed in Table 2.

Range analysis revealed that sucrose concentration exerted the strongest effect on pollen germination (R = 29.28%), followed by pH and boric acid concentration (R = 9.60% and 8.81%), while calcium nitrate concentration produced the minimum influence (R = 2.10%) (Table 2).

Based on the mean germination rates of all factor levels, sucrose at the third level (S_3) had the highest value of 86.74%, which was higher than those at the first (S_1) and second (S_2) levels. The second levels of boric acid (B_2) and pH (P_2) achieved the maximum germination rates of 77.40% and 78.66%, respectively, exceeding their corresponding first (B_1, P_1) and third (B_3, P_3) levels. For calcium nitrate, the first level (C_1) presented the highest germination percentage (74.84%), which was superior to the second (C_2) and third (C_3) levels. In general, distinct differences in pollen germination were observed among various levels of each tested factor, as reflected by the K values listed in Table 2.

Table 2

The range analysis of germination rates using an OATS $L_{Combinations}$ ($Levels^{Factors}$) = L_9 (3^4) for *F. chinensis* pollen on different media

Combinations	Factors				Germination rate (%)				
	S (sucrose) ($g \cdot L^{-1}$)	B (boric acid) ($g \cdot L^{-1}$)	C (calcium nitrate) ($g \cdot L^{-1}$)	P (pH)	Replicate I	Replicate II	Replicate III	Replicate IV	Average
1	S_1	B_1	C_1	P_1	47.06	46.81	53.06	51.04	49.49
2	S_1	B_2	C_2	P_2	67.68	68.81	64.65	62.63	65.94
3	S_1	B_3	C_3	P_3	54.46	56.44	60.19	56.70	56.95
4	S_2	B_1	C_2	P_3	66.67	72.55	72.00	68.75	69.99
5	S_2	B_2	C_3	P_1	73.53	75.00	74.23	77.23	75.00
6	S_2	B_3	C_1	P_2	84.16	84.21	86.87	79.80	83.76
7	S_3	B_1	C_3	P_2	84.85	87.25	85.87	87.13	86.28
8	S_3	B_2	C_1	P_3	90.10	93.94	89.90	91.09	91.26
9	S_3	B_3	C_2	P_1	81.52	83.16	85.86	80.20	82.69
K_1	689.53	823.04	**898.04**	828.70					
K_2	915.00	**928.79**	874.48	**943.91**					
K_3	**1040.87**	893.57	872.88	872.79					
	57.46	68.59	**74.84**	69.06					
	76.25	**77.40**	72.87	**78.66**					
	86.74	74.46	72.74	72.73					
R	29.28	8.81	2.10	9.60					

K_i denotes the sum of observed values for different levels of a single factor, denotes the mean of observed values at respective levels of a single factor, and R is the range difference between the max and min values. Three levels of sucrose (S): S_1 = 50 $g \cdot L^{-1}$, S_2 = 100 $g \cdot L^{-1}$, S_3 = 150 $g \cdot L^{-1}$; boric acid (B): B_1 = 0.05 $g \cdot L^{-1}$, B_2 = 0.1 $g \cdot L^{-1}$, B_3 = 0.2 $g \cdot L^{-1}$; calcium nitrate (C): C_1 = 0.05 $g \cdot L^{-1}$, C_2 = 0.1 $g \cdot L^{-1}$, C_3 = 0.2 $g \cdot L^{-1}$; and pH (P): P_1 = 5, P_2 =6, P_3 = 7, were performed. Values marked in bold represent the maximum data points.

Furthermore, multiple comparisons revealed that sucrose concentration, boric acid dosage and pH all significantly affected pollen germination of *F. chinensis* (p = 0.01, Table 3). Pollen germination rates differed markedly across sucrose treatments, with the maximum value of 86.74% at 150 $g \cdot L^{-1}$ sucrose. Boric acid also strongly altered pollen germination: the 0.1 $g \cdot L^{-1}$ treatment reached the highest rate (77.40%), followed by 0.2 $g \cdot L^{-1}$, whereas 0.05 $g \cdot L^{-1}$ gave the lowest germination. Similarly, the pH gradient exhibited a similar trend to boric acid treatments; pH 6 yielded the optimal germination, while pH 5 produced the minimum rate. Calcium nitrate concentration exerted no significant effect on pollen germination, even though the highest germination was observed at 0.05 $g \cdot L^{-1}$.

Table 3
The multiple comparison (S-N-K) of germination rates (%) of *F. chinensis* pollen at different levels of each factor

Levels	Sucrose (S) g·L^{-1}	Boric acid (B) g·L^{-1}	Calcium nitrate (C) g·L^{-1}	pH
1	57.46 ± 7.46C	68.59 ± 15.88C	74.84 ± 19.14	69.06 ± 14.98C
2	76.25 ± 6.36B	77.40 ± 11.11A	72.87 ± 7.84	78.66 ± 9.71A
3	86.74 ± 4.05A	74.46 ± 13.16B	72.74 ± 12.72	72.73 ± 14.92B

The different capital letters in the same column indicate a difference at the significant level of $p = 0.01$. the same applies below.

Collectively, the present results indicated that the optimal medium formulation for *in vitro* germination of *F. chinensis* pollen consisted of 150 g·L^{-1} sucrose, 0.1 g·L^{-1} boric acid, 0.05 g·L^{-1} calcium nitrate, pH 6.0 and 0.8 g·L^{-1} agar. The germination percentage of fresh *F. chinensis* pollen cultured on this optimized medium was determined across four biological replicates, with an average value of 92.62%, verifying the high reliability of the screened medium (Table S1). This 92.62% value was defined as the initial pollen germination rate of *F. chinensis.*

3.2 Impacts of culture temperature and time on pollen *in vitro* germinability

Based on the optimized medium, pollen germination of *F. chinensis* was assessed across different temperatures (5, 10, 15, 20, 25 and 30℃) and incubation durations (3, 6, 12, 24 and 48 h). At the same temperature, the *in vitro* germination rate of *F. chinensis* pollen increased with incubation time and peaked at 48 h (Table 4). For the same incubation duration (48 h), the germination rate of *F. chinensis* pollen increased gradually with rising temperature and peaked at 25℃ (92.41%) *in vitro*. Subsequently it declined at 30℃. Furthermore, when pollen was cultured at 25°C for 3 to 48 hours, its germination rate fluctuated from 52.57% to 92.41% (Table 4, Figure S1). These results indicated that incubation at 25℃ for 48 h was optimal for *in vitro* germination of *F. chinensis* pollen.

Table 4
The multiple comparison (S-N-K) of germination rates (%) for different culture times of *F. chinensis* pollen at different temperature

Time	Culture temperature (℃)					
(h)	5	10	15	20	25	30
3	1.57 ± 0.60D	3.74 ± 1.50E	38.97 ± 1.71E	49.44 ± 1.70E	52.57 ± 1.08D	56.75 ± 1.36D
6	4.61 ± 1.09D	29.73 ± 2.51D	50.89 ± 1.91D	67.76 ± 2.08D	76.38 ± 1.11C	75.56 ± 1.40C
12	20.00 ± 2.66C	35.98 ± 1.94C	57.08 ± 1.78C	75.24 ± 1.77C	84.62 ± 1.33B	82.36 ± 1.10B
24	34.44 ± 1.61B	45.30 ± 2.58B	70.98 ± 2.69B	84.22 ± 1.62B	90.36 ± 0.93A	85.23 ± 1.18A
48	41.62 ± 2.73A	51.89 ± 3.10A	76.32 ± 1.89A	88.32 ± 0.99A	92.41 ± 1.31A	87.25 ± 1.35A

3.3 Impacts of unfreezing and rehydration treatments on viability restoration of stored pollen

Viability of stored pollen was determined following thawing and rehydration treatments. The data (Table 5) indicated that pollen germination rate rose sharply with extended thawing time. At the same thawing duration, the germination rate increased gradually as rehydration time increased. The highest germination rate (91.49%) was obtained with 24 h thawing combined with 4 h rehydration. Overall, rehydration after thawing effectively improved the germinability of stored pollen.

Table 5
The effect of different unfreezing and rehydration times on pollen germination of *F. chinensis*

Unfreezing time at 4℃ (h)	Rehydration time at 25℃ (h)	Germination rate (%) Mean ± SD
0	0	74.84 ± 2.89C
12	0	79.02 ± 2.50C
12	2	83.85 ± 1.38B
12	4	86.70 ± 2.12AB
24	0	84.71 ± 2.72B
24	2	89.85 ± 1.50A
24	4	91.49 ± 1.44A

3.4 Influences of storage temperature and storage period on viability of pollen

Pollen germination rate was calculated under various storage temperatures (25, 4, -20 and − 80℃). The *in vitro* pollen germination rate of *F. chinensis* was 92.62% prior to low-temperature storage. Results (Table 6, Fig. 2) indicated that at 25℃, the pollen germination rate rapidly decreased to 35.06% at 7 d, and pollen completely lost germinability after 14 d. When stored at 4℃, the germination rate declined gradually during 14 d (67.28%), then dropped sharply from 30 d (49.51%) to 60 d (27.78%). For pollen stored at -20℃, the germination rate decreased gradually from 7 d (90.21%) to 180 d (75.07%). A sharp decline was observed from 180 d to 365 d (51.86%). At -80℃, the germination rate decreased slowly between 7 d (91.49%) and 180 d (81.92%). Subsequently, it gradually decreased to 71.16% at 365 d. These results show that pollen of *F. chinensis* can be stored short-term at 25℃ and 4℃, and kept for long period at -20℃ and − 80℃ with high viability maintained.

Table 6
Effects of storage temperature and time on pollen germination rate of *F. chinensis*

Storage time (d)	Storage temperature (℃)			
	25	4	-20	-80
0	92.62 ± 1.31A	92.62 ± 1.31A	92.62 ± 1.31A	92.62 ± 1.31A
7	35.06 ± 1.69B	78.14 ± 2.17B	90.21 ± 1.08A	91.49 ± 1.44A
14	0.00 ± 0.00C	67.28 ± 2.06C	85.84 ± 1.83B	89.23 ± 1.65AB
30	0.00 ± 0.00C	49.51 ± 2.27D	84.17 ± 1.61B	86.68 ± 1.13BC
60	0.00 ± 0.00C	27.78 ± 3.01E	80.42 ± 2.54C	84.73 ± 3.16CD
180	0.00 ± 0.00C	0.00 ± 0.00F	75.07 ± 0.81D	81.92 ± 2.06D
365	0.00 ± 0.00C	0.00 ± 0.00F	51.86 ± 2.95E	71.16 ± 3.52E

4. Discussion

Pollen viability is an important indicator reflecting plant fertility. Exploring the *in vitro* pollen viability of *F. chinensis* provides technical support for cross breeding and helps improve breeding efficiency. The *in vitro* germination method is an effective approach to detect pollen viability (Althiab et al., 2024). Medium composition exerts a remarkable effect on *in vitro* pollen germination rate (Kato et al., 2025). Sucrose, boric acid and Ca^{2+} are key factors regulating pollen germination *in vitro* (Liu et al., 2020; Impe et al., 2020). Sucrose supplies energy for pollen tube elongation and maintains osmotic pressure to prevent pollen cells from swelling and rupturing (Barinova et al., 2023). Boric acid facilitates sugar transport, stabilizes cell membranes and strengthens pollen walls (Fragallah et al., 2022). An appropriate concentration of Ca^{2+} promotes pollen germination and pollen

tube growth (Hirose et al., 2014). In addition, pH is another critical factor for *in vitro* pollen germination. A suitable pH value ensures normal transmembrane transport of substances and enhances Ca^{2+} uptake (Mortazavi et al., 2022). In this study, orthogonal experiments were conducted to determine the optimal medium components for *F. chinensis* pollen culture (Table 1, Fig. 1): 150 $g{\cdot}L^{-1}$ sucrose, 0.1 $g{\cdot}L^{-1}$ boric acid, 0.05 $g{\cdot}L^{-1}$ calcium nitrate, and pH 6.0. Imbalanced concentrations of sucrose and boric acid, excessive Ca^{2+} content and inappropriate pH values all inhibit pollen germination and pollen tube growth. These findings are consistent with previous reports (Liu et al., 2020; Impe et al., 2020). When cultured on the optimized medium, the *in vitro* germination rate of *F. chinensis* pollen exceeded 90%, indicating high pollen viability (Table S1). This lays a solid foundation for cross breeding among different clones and varieties of *F. chinensis*.

Incubation temperature plays a crucial role in pollen germination, and appropriate temperatures are conducive to pollen development (Rosbakh et al., 2016). For instance, the optimal temperature for *in vitro* pollen culture of rice is 27℃, with a germination rate of 85%, while both excessively high and low temperatures lead to lower germination rates (Coast et al., 2016). The optimum temperature for *in vitro* pollen germination of banana is 28℃ (Zhang et al., 2023). In this study, germination was slow at low temperatures, rose to a maximum at 25℃, and then steadily decreased with further temperature increases. Accordingly, 25℃ was identified as the optimal germination temperature (Figure S1, Table 4).

Thawing and rehydration are critical steps to improve the *in vitro* germination rate of pollen preserved under long-term low-temperature storage, which has been verified in numerous plant species (Conner, 2011; Machado et al., 2014; Wang et al., 2021). For *Paeonia ostii*, pollen stored at low temperature for 300 days shows a germination rate of 77.28% after 30 min of thawing and 30 min of rehydration (Li et al., 2023). In coconut, the survival rate of frozen pollen samples reached 81.93% after thawing at 4℃ for 2 h followed by 2 h of rehydration (Machado et al., 2022). Similarly, our study revealed that frozen *F. chinensis* pollen achieved an *in vitro* germination rate of 91.24% after 24 h of thawing at 4℃ followed by 4 h of rehydration at 25℃, confirming that thawing and rehydration can effectively restore the germination capacity of stored pollen (Table 5).

As a key procedure for cross breeding, low-temperature storage can markedly prolong pollen longevity. Previous studies have demonstrated that the germination capacity of apple pollen stored at 4℃ for 7 days is five times higher than that stored at room temperature (Ćalić et al., 2021). For pollen of eight *Rosaceae* species, the maximum *in vitro* germination rate was observed after four months of storage at -20℃ (Beltrán et al., 2019). In addition, soybean pollen retained a germination rate of approximately 90% after one year of storage at -80℃ (Jia et al., 2022). This study showed that *F. chinensis* pollen could not be stored at 25℃ and 4℃ for more than 7 and 60 days; otherwise, the germination rate dropped to a very low level. When stored at -20℃ and − 80℃ for 365 days, the pollen germination rates remained 51.86% and 71.16%, respectively, demonstrating that both low temperatures can maintain high pollen viability over the long term (Table 6). Our findings provided guidelines for preserving pollen in the applied breeding of *F. chinensis*.

5. Conclusion

In summary, the optimal medium for in vitro pollen germination of *F. chinensis* consisted of 150 $g{\cdot}L^{-1}$ sucrose, 0.1 $g{\cdot}L^{-1}$ boric acid, and 0.05 $g{\cdot}L^{-1}$ calcium nitrate at pH 6.0. It was found that 25℃ was the favorable temperature for pollen germination of *F. chinensis*. In addition, pollen grains were suitable for short-term storage at 25℃ and 4℃, while long-term storage at -20℃ and − 80℃ could facilitate pollination for cross-breeding in the next growing season. Meanwhile, thawing at 4℃ for 24 h and rehydration at 25℃ for 4 h significantly improved pollen germination capacity, achieving the maximum in vitro germination rate. This study establishes a full system to boost *F. chinensis* pollen germination and fertility and provides practical guidelines for pollen preservation in breeding.

Declarations

The authors declare no conflict of interest.

Author contribution

H.S. Investigation, data curation, formal analysis, software, writing-original draft; H.Y.G. Supervision, writing-review & editing; X.Y.J. Resources, funding acquisition; Y.L.W. Conceptualization, methodology, reference collection and collation.

Data availability

All relevant data are presented within the paper and its supplementary files.

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Figures

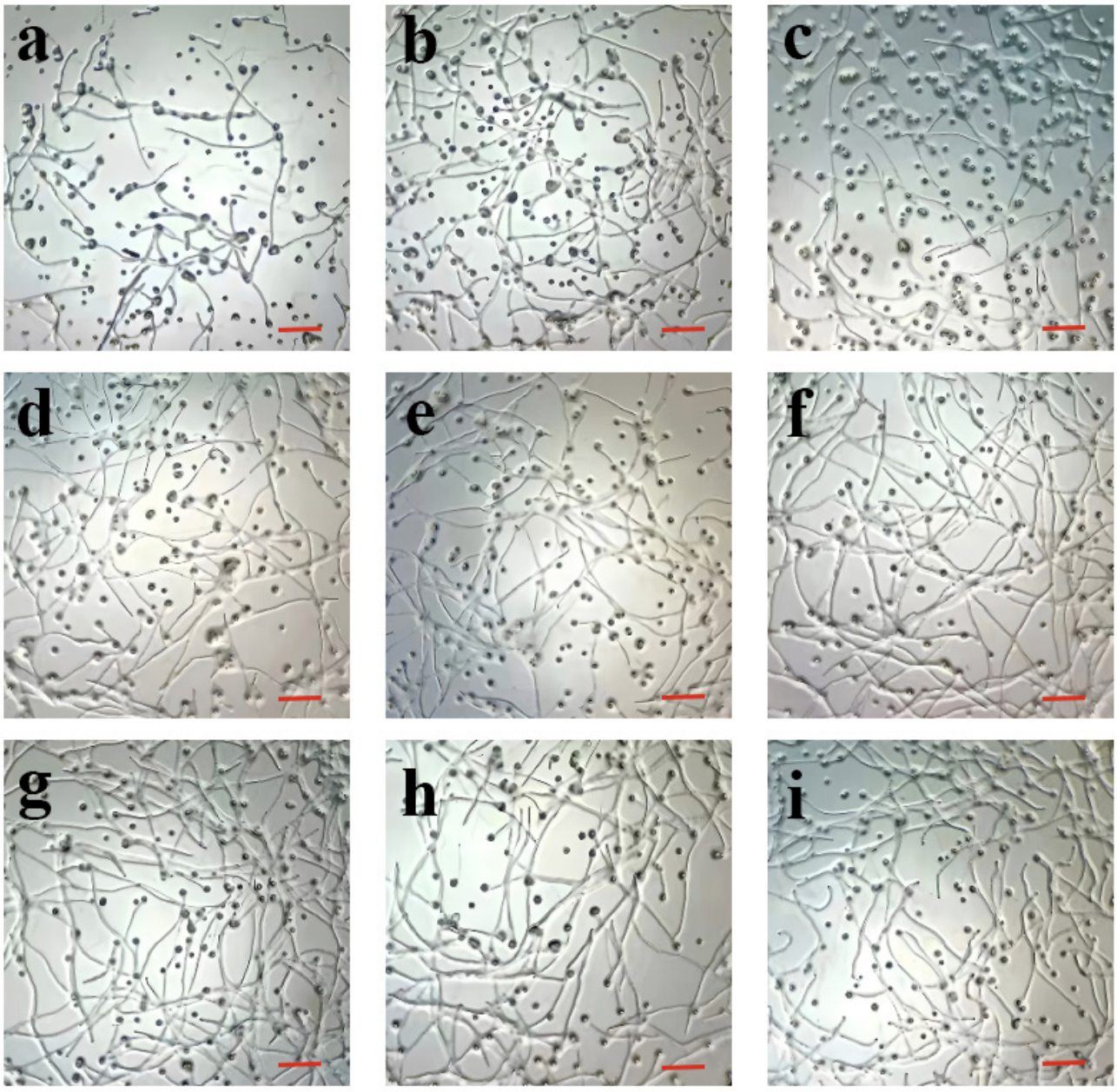


Figure 1

***In vitro*germination of *F. chinensis* pollen on Culture media containing different components.** Letters a–i represent different medium components used in the orthogonal experiment in Table 2. Scale bar represents 100 μm.

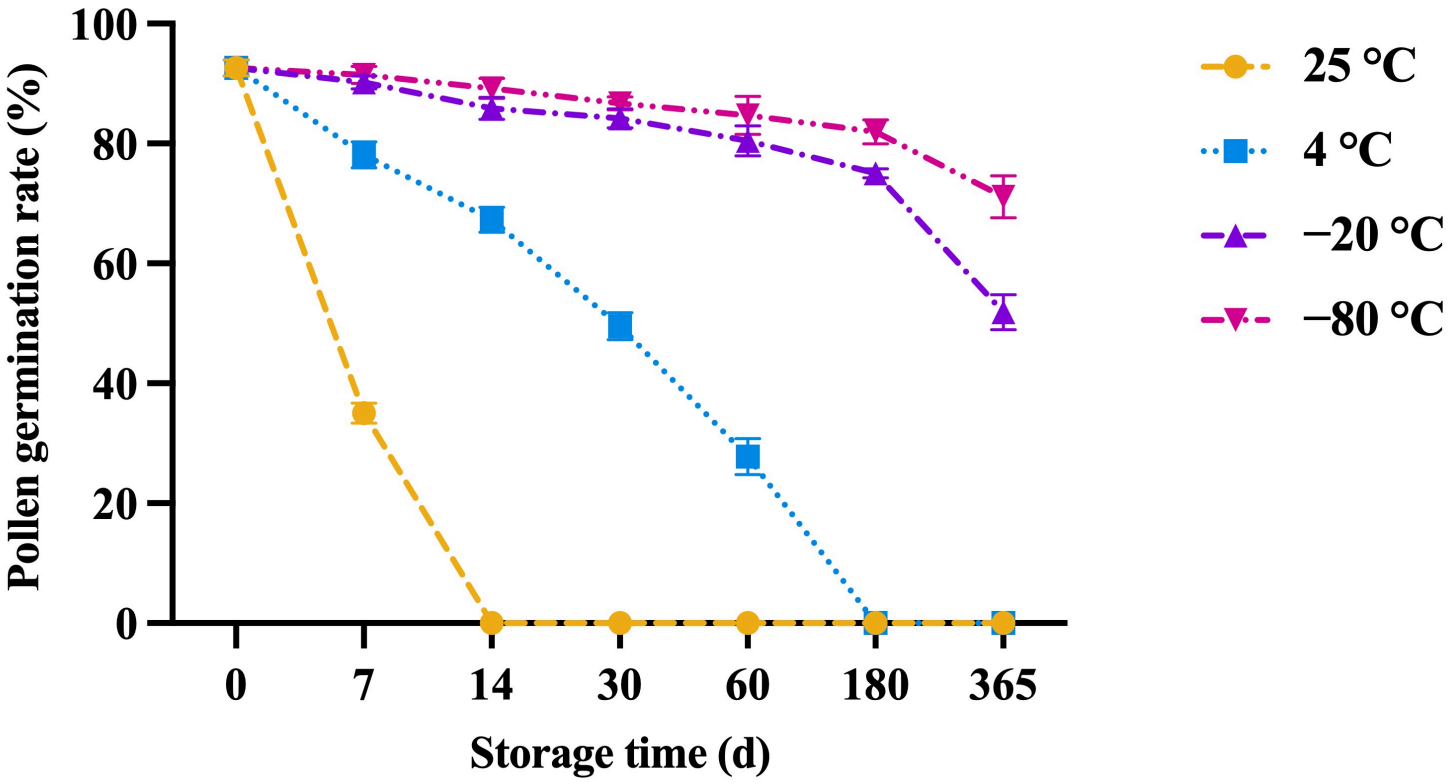


Figure 2

Germination rates (%) of *F. chinensis* pollen at different storage temperatures and times.

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- SupplementaryMaterials8.18.docx