# COMPARATIVE STUDY ON THE BIOLOGICAL CHARACTERISTICS OF POLLEN VIABILITY, POLLEN GRAIN MORPHOLOGY AND STIGMA RECEPTIVITY OF EIGHT EARLY-SPRING FLOWERING PLANTS

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**Abstract.** Flowering phenology and biological characteristics of 8 early-spring plants belonging to different genera under the same growth environment were observed and studied in this experiment in china, and compared and analyzed the pollen vitality of plants using TTC staining, red ink staining and sucrose solution germination methods, the flower pollen morphology was observed using an optical microscope, and the number of pollen grains was calculated using a scanning electron microscope. Benzidine-hydrogen peroxide method was used to detect the stigma receptivity of plants. The objective was to investigate the differences of pollen viability, stigma acceptability, flowering phenology and seed setting rate of 8 different plants in the same environment. The results showed that the 8 species had different plant heights and crown widths, longer flowering periods, similar single flower structures and abundant inflorescence types. The number of pollen grains ranged from 54,200 to 109,680. After testing the pollen viability and stigma receptivity, it was found that the pollen viability and stigma receptivity of *Hypecoum erectum* L. were the weakest. The red ink staining method was not applicable to the pollen viability detection of *Rhododendron mucronulatum* Turcz., *Forsythia suspensa* (Thunb.) Vahl and *Prunus sibirica* L.

**Keywords:** pollen, pollen grain number, TTC staining method, benzidine-hydrogen peroxide method, electron microscope scanning method

## Introduction

As an important carrier of genetic information, pollen is an indispensable material for plants in genetic reproduction, hybrid breeding and germplasm preservation (Zhao et al., 2019a). Compared with other plant tissues and organs, pollen morphology is more stable, and the rich information contained in pollen can provide a strong basis for the study of plant taxonomy and systematics (Wang et al., 2016). Shen et al. (2006) found that the pollen grains of Vaccinium ashei have a tetrahedral shape, medium size, Yang et al. (2003) found that the pollen grain morphology of 4 plants in the Papaveraceae Juss. were different. The research on Rosaceae Juss. mainly focuses on the seed dormancy characteristics, the determination of plant extracts and the investigation of germplasm resources (Li et al., 2023; Xiao et al., 2024; Liu et al., 2024). The research on the Oleaceae Hoffmanns. & Link has been limited to the study of the plant's internal chemical content and its resistance to frost (Tu et al., 2007; Xu et al., 2007). The main focus of research on Caprifoliaceae at present is on the extraction of DNA and issues related to their application in landscaping (Liu et al., 2005; Hao et al., 2006). Pollen from different plant species often develops unique morphological characteristics during the long-term evolution process. Even pollen from the same species has morphological diversity, it less affected by external environmental conditions, and has strong heritability and stability (Zhang et al.,


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2016). During the evolution and development of plants, there is no obvious change in the shape of each pollen, pollen wall patterns, the number and position of germination pores, etc., which can objectively reflect the diversity among species. Studying pollen morphological characteristics is of great significance for understanding plant evolution, relationship and plant classification.

Pollen viability refers to the ability of pollen to survive, grow, germinate or develop. It is an important basis for judging pollen quality and has a direct impact on pollination, fertilization, seed yield and quality (Li, et al., 2004). Vigorous pollen is one of the important factors for plant reproductive success and sexual reproduction, and is also an important research topic in plant reproductive biology (Cai, et al., 2023). In the process of sexual reproduction, the mismatch of flowering period is a difficult problem that needs to be faced. Understanding the changes in pollen vitality and storage properties is of great significance for solving the temporal and spatial barriers and parent selection problems in the process of sexual reproduction (Liu, et al., 2011). The technology of pollen vitality detection has also become an important task in carrying out plant hybrid breeding. The commonly used methods for determining pollen viability are in vitro germination and staining. The staining method uses the color change caused by the reaction of certain enzymes in the cells with specific compounds to determine the activity of the enzyme. This method takes a short time to operate, but is easily affected by the characteristics of the pollen itself and causes errors. Therefore, for different types of plants, it is necessary to explore appropriate staining methods to determine pollen vitality (Liang, 2022).

Stigma receptivity is a prerequisite for successful plant reproduction and is affected by factors such as the length of a single flower, the time after flowering, and stigma secretions (Zhang et al., 2022). The degree of stigma receptivity plays a vital role in plant sexual reproduction and directly determines whether the fertilization process can be completed (Su et al., 2016). The stronger the receptivity of the stigma, the higher its ability to accept viable pollen and maintain pollen germination. The receptive stigma is a natural culture medium for pollen, which can provide material support for pollen adhesion, germination and pollen tube growth (Sanzol et al., 2003). In nature, pollination is the basis of plant reproduction, and pollen grains must reach suitable receptive stigmas when they are still vigorous (Li et al., 2021). Therefore, stigma receptivity is an essential research topic in the process of plant reproduction.

Early-spring flowering plants are generally perennial plants that grow under deciduous broad-leaved forests. They bloom when the ice and snow melt, then unfold their leaves and produce fruits. They have significant ecological value and high economic value. They are widely distributed in northeast China, such as Jilin Province and Heilongjiang Province (Zhang et al., 2023a). The phenological characteristics of early-spring plants are unique, and the floral characteristics, pollination ability and seed setting rate reflect their growth and development capabilities at present, research on early-spring plants mainly focuses on physiological research under the influence of flowering period prediction, litter decomposition, nitrogen increase and water reduction, etc. (Gao et al., 2022; Fang et al., 2021). There are few reports on the phenological biological characteristics, pollen characteristics, pollen viability, stigma receptivity and seed setting rate of early-spring plants. Therefore, this study selected 8 early-spring plants with similar flowering time, ornamental value and the same growth environment in northern China, and carried out detailed research and analysis on their flowering phenology, pollen quantity and morphology, pollen viability, stigma acceptability and


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seed setting rate. To understand the difference of reproductive ecological characteristics of plants of different families and genera under the same growth environment, and then provide some reference for the protection, development and utilization of plant resources in early spring.

## Study area and introduction of materials

### *Study area*

The experimental materials were selected from Changchun Baihua Garden (44°21′ N, 125°28′ E) located in Changchun City, Jilin Province, China. The plants in the garden belong to 68 families, 172 genera, and 216 species, with a temperate continental semi-humid climate. During the study of plant phenology, the lowest temperature was in March, which could reach -4°C, and the highest temperature was in mid-June, which could reach 26°C; The lowest precipitation is 2 mm in early April and the highest is 17 mm in mid-June (*Fig. 1*). The frost-free period is 140-150 d per year, and the freezing period is 5 months per year. The soil type is mainly black soil with a pH value of 7.0-9.0 (Zhang, 2005; Chen et al., 2011).

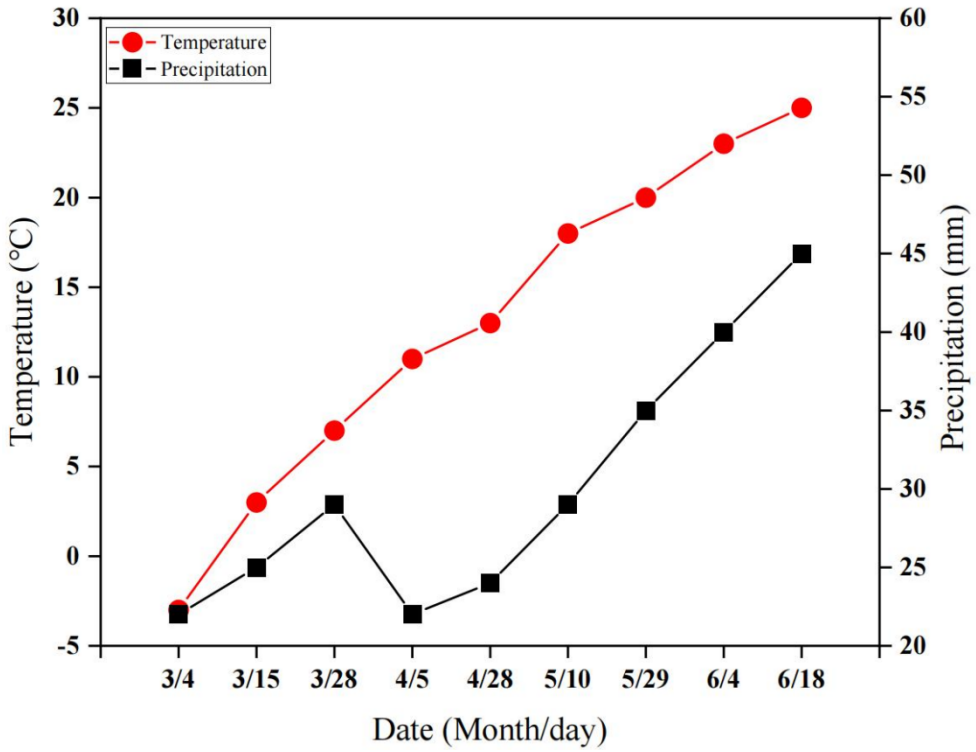


***Figure 1.*** *Changes of temperature and precipitation in the study area during the phenological periods of 8 early-spring plants (Data source: The Meteorological Station of Changchun, Jilin Province, China)*

### *Materials*

The experimental research materials are 8 common flowering ornamental plants in Northeast China in Changchun Baihua Garden. Of these, 7 species are perennial plants, *Hypecoum erectum* L. is an early-spring ephemeral plant that completes its life cycle


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along with 7 other plants each spring. They belonging to *Spiraea* L. of Rosaceae Juss., *Syringa* Linn. of Oleaceae Hoffmanns. & Link, *Forsythia* Vahl of Oleaceae Hoffmanns. & Link, *Prunus* L. of Rosaceae Juss., *Rhododendron* L. of Ericaceae Juss., *Sambucus* L. of Caprifoliaceae Juss., *Armeniaca* Mill. of Rosaceae Juss., and *Hypecoum* L. of Papaveraceae Juss., they are *Spiraea thunbergii Siebold* ex Blume (Tian et al., 2007), *Syringa oblata* Lindl. (Lu et al.,2003), *Forsythia suspensa* (Thunb.) Vahl (Zhao et al., 2019b), *Prunus padus* L. (Zhu et al., 2005), *Rhododendron mucronulatum* Turcz. (Sun et al., 2024), *Sambucus williamsii* Hance (Liao et al., 2004), *Prunus sibirica* L. (Diao, 1994), and *Hypecoum erectum* L. (Cai et al., 2007) (*Fig. 2*).


***Figure 2.*** *8 early-spring plants for research materials. (A) H. erectum (Hypecoum erectum L.). (B) P. Sibirica (Prunus sibirica L.). (C) S. oblata (Syringa oblata Lindl.). (D) R. mucronulatum (Rhododendron mucronulatum Turcz.). (E). F. suspensa (Forsythia suspensa (Thunb.). Vahl) (F) P. padus (Prunus padus L.). (G) S. thunbergii (Spiraea thunbergia Siebold ex Blume). (H) S. williamsii (Sambucus williamsii Hance)*

## Research methods

### *Research on phenology and biological characteristics*

The flowering process of 8 early-spring plants was observed at 4 levels: group, inflorescence, individual and single flower from May to June 2023. 30 well-developed single flowers were randomly collected at the peak flowering period for anatomical observation, and the crown width and plant height of the plants were measured with a tape measure. The flowering period of 10 inflorescences at the peak of flowering of 50% or more of the selected plants was recorded, and the single flower structure and inflorescence type were observed regularly every day. At the population level, 25% of the plants were in the initial flowering stage, 50% and above of the plants were in the peak flowering stage, and 95% of the plants were in the final flowering stage; At the inflorescence level, the opening day of the first flower is the beginning of flowering, the peak flowering period of 50% or more of the plants, and the end of flowering period when all flowers fall off; At the individual level, the date when the first inflorescence in an individual blooms is the beginning of flowering for that individual, the date when 50% of the inflorescences in an individual bloom is the peak of flowering for that individual, and the date when the last inflorescence in an individual finishes blooming is the final flowering for that individual; At the single flower level,


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20 flower buds were selected from 60 inflorescences and observed every day from the beginning of bud formation, with an interval of 2-3 h until fruiting, and the duration of flowering was recorded. The flowering synchronization index was calculated based on the above data.

$$S_i = \frac{1}{n-1}\left(\frac{1}{f_i}\right)\sum_{j=0}^{n} e_{j \neq i} \quad \text{(Eq.1)}$$

$e_i$ is overlapping time of flowering period between individuals i and j (d); $f_i$ is the duration of flowering of individual i (d); n is the total number of individuals, $0 \leq S_i \leq 1$ (0 means no overlapping flowering period, 1 means complete overlapping flowering period).

### *Pollen grain count*

Before the experiment, the flower buds of the 8 species were processed. For each variety, 90 unopened flower buds were taken, the anthers were peeled off, and they were evenly placed in 3 clean penicillin vials and dried naturally. After the pollen was fully dispersed, 1 mL of 1% sucrose solution was dripped into each bottle, and the volume was adjusted to 2 mL. The bottle was covered and shaken to make the pollen suspended. Use a 20 μL microsampler to draw 1 drop of the suspension onto a hemocytometer with a coverslip, and count the pollen grains in the field of view under a LEO-1430VP low-power microscope (10-40× magnification). Repeat 6 times for each plant and 6 times for each vial, take the average value, and calculate the number of pollen grains in a single anther (N).

$$N = (\text{average number of pollen in the field of view} \times 10000 \times 2) / 30 \quad \text{(Eq.2)}$$

### *Observation of pollen grain morphology*

Dried pollen grains of 8 plants were attached to the double-sided adhesive tape on the sample stage of JSM-6510LA scanning electron microscope, and the equatorial and polar morphologies of the pollen and the outer wall patterns of the equatorial plane were observed. The polar axis length, equatorial axis length and germination groove length of the pollen grains were measured.

### *Pollen vitality test*

The experimental materials were collected at 7:30 am on a sunny day. Anthers were removed from the 8 plants at 0, 2, 4, 6, 8, 10, 12, and 14 d after flowering, and the flowers were covered with seed bags and the anthers were brought back to the laboratory. After full flowering, the anthers were removed and the pollen grains were brushed off the anthers with a disposable small brush in time.

### *TTC staining*

Add 1 to 2 drops of 0.5% TTC solution into a centrifuge tube to disperse the pollen in the solution. Place the centrifuge tube in a 120°C oven for 20 min. Take it out and use a pipette to absorb the pollen suspension onto a glass slide. Observe it under an optical microscope (BM 2000). Viable pollen is red, slightly vigorous pollen is light red, and inactive or sterile flowers are colorless.


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### *Sucrose solution germination method*

Dip a small amount of pollen with a brush and place it on a glass slide, then drop 1-2 drops of sucrose solution on each glass slide. Place the glass slide in a culture dish lined with wet filter paper, and place the glass slide in an artificial climate incubator at 35°C for 48 h. Then, observe the glass slide under an optical microscope (BM2000). When the number of pollen grains in each field of view is greater than or equal to 50 and the length of the pollen tube is greater than the diameter of the pollen, it can be considered to have germinated.

### *Red ink staining*

Prepare 5% red ink solution, drop 1 to 2 drops on a glass slide, take a small amount of pollen, stir evenly and cover with a coverslip, and examine under a microscope immediately. Viable pollen will not be stained, while inactive pollen will be stained red.

### *Stigma receptivity test*

The benzidine-hydrogen peroxide method was used to determine the receptivity of stigmas. The stigmas of flowers of 8 plants at 0 d, 2 d, 4 d, 6 d, 8 d, 10 d, 12 d, and 14 d after flowering were placed in a culture dish containing benzidine-hydrogen peroxide reaction solution (1% benzidine: 3% hydrogen peroxide: water = 4:11:22) so that the stigmas were completely submerged in the reaction solution for 5 min. The stigmas were then observed under an optical microscope (BM2000). If the area around the stigma was blue and accompanied by a large number of bubbles, the stigma was receptive. The degree of receptivity is determined by the number of bubbles produced by the reaction liquid around the stigma. If the receptivity of the stigma is strong, a large number of bubbles will be produced in the reaction liquid around the stigma. Conversely, if the receptivity of the stigma is weak, fewer bubbles will be produced. The grading standard is: "+"represents there are a few bubbles, the stigma receptivity is weak; "++" represents there are more bubbles, the stigma receptivity is medium; "+++" represents there are a lot of bubbles, the stigma receptivity is strong; "–" represents there are no bubbles, the stigma receptivity is lost. The sampling time point of the stigma is the same as the sampling time point of the pollen.

### *Seed setting test*

100 inflorescences were randomly selected from each of the 8 plant populations, and the maturity time of individual capsules was recorded (when the capsule was green, it was recorded as the start time of capsule maturity, and when the capsule was yellow, it was recorded as the end time of capsule maturity). At the same time, the number of fruits and buds on each inflorescence was counted, and the seed setting rate was calculated.

$$\text{Seed setting rate} = \text{number of seeds} / \text{number of buds} \times 100\% \quad \text{(Eq.3)}$$

### *Data analysis*

The data were preliminarily processed using Excel 2019. The plots were drawn using Origin 2021. One-way ANOVA was used in SPSS to conduct a significance analysis on the number of pollen grains and pollen vitality of different types of early- spring plants.


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## Results

### *Research on phenological characteristics*

Through the observation and statistics of the flowering phenology of the plants, the flowering period of the 8 species ranged from mid-March to mid-to-late June. The average duration of individual flowering was 28 d, of which the *S. williamsii* had the longest duration of 46 d, and the *F. suspensa* had the shortest duration of 15 d. The individual flowering synchronization index of *R. mucronulatum* was the highest, which was 0.723; the individual flowering synchronization index of *P. padus* was the highest, which was 0.564 (*Eq. 1*). The plant heights of the 8 species ranged from 30 to 2400 cm. There is a highly significant difference between *P. padus* and *H. erectum* ($p < 0.01$), a significant difference between *S. williamsii* and *R. mucronulatum* ($p < 0.05$), and no significant difference between *S. oblata* and *P. sibirica* ($p > 0.05$). The crown width ranges from 7 to 750 cm, with the (east-west) of crown width of *H. erectum* being 9 cm and the (south-north) of crown width being 12 cm, which is the smallest crown width. The crown width (east-west) of *P. padus* is 600 cm, and the crown width (south-north) is 720 cm, which are the maximum crown widths. There is a significant difference between *P. padus* and *P. sibirica* ($p < 0.05$), and a highly significant difference between *P. padus* and *S. thunbergii* ($p < 0.01$); there is a significant difference between *S. oblata* and *F. suspensa* ($p < 0.05$). All single flower structures are single-petal flowers, and the number of stamens in *P. sibirica* is the largest, ranging from 20 to 45; *F. suspensa* and *S. oblata* have the least number of stamens, both with two. There are 4 types of inflorescence: panicle, raceme, umbel and cyme. The duration of single flower opening of the 8 species ranged from 20 to 80 d, with *F. suspensa* having the longest duration and *P. sibirica* having the shortest (*Table 1*).

### *Number of pollen grains*

The anther pollen number of *P. padus* is the largest, followed by *S. oblata*, and the anther pollen number of *S. thunbergii* is the smallest, about 1/2 of that of *P. sibirica*. The pollen counts of *F. suspensa*, *P. padus*, and *S. williamsii* were $86{,}000 \pm 2{,}253$, and those of *R. mucronulatum* and *H. erectum* were $74{,}000 \pm 2{,}147$ (*Eq. 2*). There is a significant difference between *P. sibirica* and *F. suspensa* ($p < 0.05$); A significant difference exists between *P. sibirica* and *P. padus* ($p < 0.05$), and an highly significant difference exists between *P. sibirica* and *S. thunbergii* ($p < 0.01$); No significant difference between *R. mucronulatum* and *H. erectum* ($p > 0.05$) (*Fig. 3*).

### *Morphological characteristics of pollen grains*

The polar axis length of pollen of the 8 species is 17.44-54.98 μm, with a smaller span, there is a significant difference between *S. oblata* and *F. suspensa* ($p < 0.05$), and a highly significant difference between *P. sibirica* and *S. thunbergii* ($p < 0.01$); the equatorial axis length of pollen is 8.16-42.56 μm, with a larger span, there is no significant difference between *P. padus* and *S. thunbergii* ($p > 0.05$), and a highly significant difference between *S. oblata* and *F. suspensa* ($p < 0.05$). The shapes of pollen grains are prolate, subspherical, and tetrad with rounded triangles. The outer wall patterns of pollen are striped-perforated, reticular, smooth, and composite. The germination organs of *R. mucronulatum* are all three-grooved except for the diffuse pores. The length of the pollen germination groove ranged from 13.56 to 49.40 μm, a highly significant difference exists between *S. thunbergii* and *P. sibirica* ($p < 0.01$), and


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an significant difference exists between *S. oblata* and *F. suspensa* ($p < 0.05$), the width of the germination groove ranged from 1.16 to 9.43 μm, a highly significant difference exists between *R. mucronulatum* and *S. williamsii* ($p < 0.01$), and no significant difference exists between *S. oblata* and *F. suspensa* ($p > 0.05$) (*Fig. 4; Table 2*).

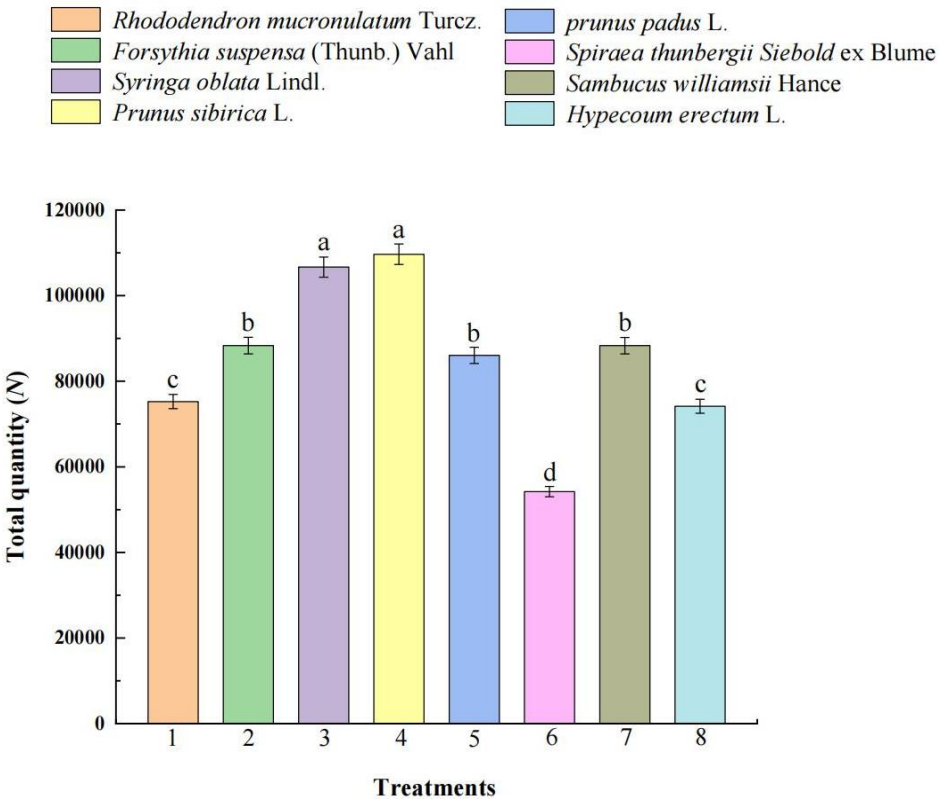


***Figure 3.*** *Number of pollen grains of 8 early-spring plants. The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions*

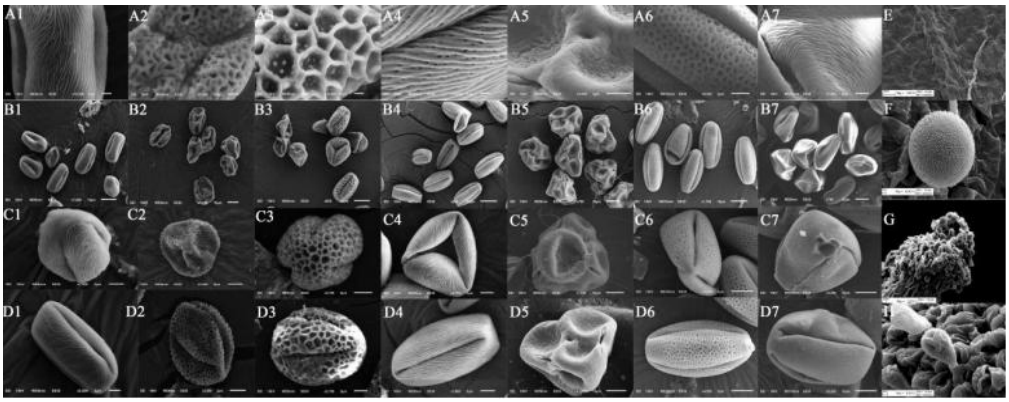


***Figure 4.*** *Scanning electron microscopy images of pollen morphology of 8 plants.* (*A*) Outer *wall ornamentation; (B) pollen grain morphology; (C) polar view; (D) lateral view. 1. S. thunbergii, Bar = 1 μm,10 μm,5 μm,2 μm. 2. S. oblata, Bar = 2 μm,10 μm,5 μm, 5 μm. 3. F. suspensa, Bar = 1 μm,20 μm,5 μm, 5 μm. 4. P. padus, Bar = 1 μm,20 μm,5 μm, 5 μm. 5. R. mucronulatum, Bar = 5 μm,20 μm,10 μm, 10 μm. 6. S. williamsii, Bar = 2 μm,10 μm,5 μm, 5 μm. 7. P. sibirica, Bar = 2 μm,20 μm,10 μm, 10 μm. 8. H. erectum, Bar = 10 mm,10 mm, 10 mm,10 m*


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***Table 1.*** *Phenological information of 8 plant species*

| Species | Height (cm) | Crown width (cm) | Single flower structure | Inflorescence type | Date of first flowering (month-day) | Flowering duration (d) | Peak flowering period (month-day) | End of flowering period (month-day) | Flowering synchronicity index |
|---|---|---|---|---|---|---|---|---|---|
| *F. suspensa* | 250 ± 50c | 200 ± 100c | Single flower, 2 stamens | Panicle | 3.11(population)<br>3.15(/individua)<br>3.17(inflorescence) | 17(population)<br>15(/individua)<br>14(inflorescence) | 3.21(population)<br>3.23(/individua)<br>3.26(inflorescence) | 3.28(population)<br>3.30(/individua)<br>3.31(inflorescence) | -(population)<br>0.624(/individua)<br>-(inflorescence) |
| *P. sibirica* | 350 ± 150bc | 325 ± 25b | Single flower, 20-45 stamens | Umbel raceme | 3.20(population)<br>3.24(/individua)<br>3.21(inflorescence) | 23(population)<br>21(/individua)<br>25(inflorescence) | 4.1(population)<br>4.8(/individua)<br>4.8(inflorescence) | 4.12(population)<br>4.14(/individua)<br>4.15(inflorescence) | -(population)<br>0.712(/individua)<br>-(inflorescence) |
| *P. sibirica* | 1400 ± 1000a | 650 ± 100a | Single flower,10 stamens | Raceme | 4.14(population)<br>4.17(/individua)<br>4.20(inflorescence) | 26(population)<br>25(/individua)<br>26(inflorescence) | 4.25(population)<br>4.26(/individua)<br>4.28(inflorescence) | 5.10(population)<br>5.12(/individua)<br>5.16(inflorescence) | -(population)<br>0.564(/individua)<br>-(inflorescence) |
| *S. oblata* | 275 ± 125bc | 110 ± 10d | Single flower,2 stamens | Panicle | 5.11(population)<br>5.13(/individua)<br>5.14(inflorescence) | 36(population)<br>36(/individua)<br>37(inflorescence) | 5.27(population)<br>5.29(/individua)<br>5.31(inflorescence) | 6.16(population)<br>6.18(/individua)<br>6.20(inflorescence) | -(population)<br>0.643(/individua)<br>-(inflorescence) |
| *S. williamsii* | 550 ± 50b | 225 ± 25c | Single flower,5 stamens | Panicle cymes | 4.7(population)<br>4.8(/individua)<br>4.9(inflorescence) | 44(population)<br>46(/individua)<br>46(inflorescence) | 4.21(population)<br>4.23(/individua)<br>4.25(inflorescence) | 5.19(population)<br>5.19(/individua)<br>5.21(inflorescence) | -(population)<br>0.632(/individua)<br>-(inflorescence) |
| *H. erectum* | 45 ± 15d | 11 ± 4e | Single flower,4 stamens | Dichotomous cyme | 4.17(population)<br>4.18(/individua)<br>4.23(inflorescence) | 38(population)<br>39(/individua)<br>36(inflorescence) | 5.20(population)<br>5.23(/individua)<br>5.25(inflorescence) | 5.25(population)<br>5.27(/individua)<br>5.29(inflorescence) | -(population)<br>0.614(/individua)<br>-(inflorescence) |
| *R. mucronulatum* | 150 ± 50c | 95 ± 45d | Single flower,10 stamens | Axillary umbels | 4.16(population)<br>4.18(/individua)<br>4.18(inflorescence) | 22(population)<br>22(/individua)<br>24(inflorescence) | 4.23(population)<br>4.25(/individua)<br>4.27(inflorescence) | 5.8(population)<br>5.10(/individua)<br>5.12(inflorescence) | -(population)<br>0.723(/individua)<br>-(inflorescence) |
| *S. thunbergii* | 175 ± 25c | 105 ± 15d | Single flower, 18-20 stamens | Umbel | 4.18(population)<br>4.20(/individua)<br>4.23(inflorescence) | 20(population)<br>19(/individua)<br>19(inflorescence) | 4.26(population)<br>4.28(/individua)<br>4.30(inflorescence) | 5.8(population)<br>5.9(/individua)<br>5.12(inflorescence) | -(population)<br>0.672(/individua)<br>-(inflorescence) |

The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions

***Table 2.*** *Comparison of pollen grain morphology of 8 plants*

| Species | Polar axis (μm) | P/E (μm) | Shape | Exine ornamentation | Apcture | Length of germination groove (μm) | Width of germination groove (μm) |
|---|---|---|---|---|---|---|---|
| *S. thunbergii* | 17.83 ± 0.40e | 1.87 ± 0.24a | Prolate spheroid | Stripe-perforated | 3 grooves | 14.95 ± 1.13e | 2.21 ± 0.16c |
| *S. oblata* | 23.37 ± 0.7d | 1.18 ± 0.15e | Nearly spherical | Coarse mesh | 3 grooves | 21.17 ± 0.51cd | 2.72 ± 0.59b |
| *F. suspensa* | 36.44 ± 0.83c | 1.42 ± 0.15cd | Prolate spheroid | Stripe-Perforated | 3 grooves | 30.46 ± 0.57b | 3.51 ± 0.61b |
| *P. padus* | 36.68 ± 0.70c | 1.92 ± 0.10a | Prolate spheroid | Net carving | 3 grooves | 30.91 ± 1.16b | 2.39 ± 0.38c |
| *R. mucronulatum* | 50.48 ± 3.44b | 1.20 ± 0.10de | The tetrahedron is a rounded triangle | Smooth | Diffuse pores | 20.31 ± 1.82d | 9.04 ± 0.29a |
| *S. williamsii* | 25.06 ± 0.31d | 1.92 ± 0.12a | Prolate spheroid | Mesh | 3 grooves | 22.29 ± 0.44c | 1.42 ± 0.22d |
| *P. sibirica* | 52.34 ± 2.19a | 1.76 ± 0.2ab | Prolate spheroid | Stripe-Perforated | 3 grooves | 47.44 ± 1.48a | 2.22 ± 0.24c |
| *S. williamsii* | 19.312 ± 0.412e | 1.53 ± 0.2bc | Nearly spherical | Composite pattern, with shorter spines | 3 grooves | 20.62 ± 1.74cd | 2.53 ± 0.36c |

The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions


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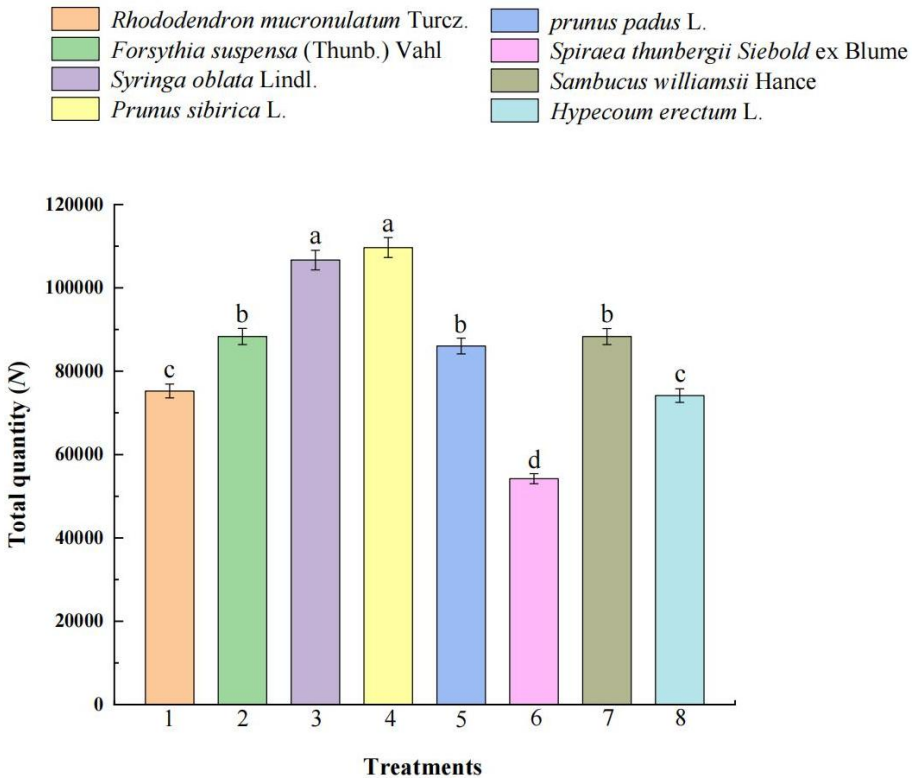


***Figure 3.*** *Number of pollen grains of 8 early-spring plants. The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions*

## *Pollen vitality*

### *TTC staining method for determination of pollen viability*

The pollen viability of *H. erectum* was high on the 0th d, reaching a peak on the 2nd d and then decreasing until there was no pollen viability after the 6th d. There was no significant differences between *S. oblata* and *F. suspensa* on 2nd d ($p > 0.05$). A significant difference was observed on the 4th d ($p < 0.05$), and a highly significant difference was presented on the remaining days ($p < 0.01$). In 0-4 d, there was a significant difference between *S. thunbergii* and *P. sibirica* ($p < 0.05$); In 6-14 d, there was a highly significant difference ($p < 0.01$). *H. erectum* and *R. mucronulatum* have a highly significant difference from *S. williamsii* ($p < 0.01$). In 6-14 d, there was a highly significant difference between *P. sibirica* and *P. padus* ($p < 0.01$) (*Fig*. 5).

### *Red ink staining method for determination of pollen viability*

At different flowering periods, the pollen viability test results of *R. mucronulatum*, *F. suspensa* and *P. sibirica* were all 100%. Therefore, the red ink staining method is not suitable for the determination of pollen viability of these 3 plants. On the 2nd, 8th, 10th, 12th, and 14th d, a significant difference was presented between *S. oblata* and *S. thunbergii* ($p < 0.05$), On 0th d and 4th d respectively, there exist extremely significant differences ($p < 0.05$) and no significant differences ($p > 0.05$). There is a highly significant difference between *S. oblata* and *H. erectum* ($p < 0.01$). On the 8th d and the 14th d, no significant difference was observed between *S. oblata* and *S. williamsii* ($p > 0.05$) (*Fig*. 6).


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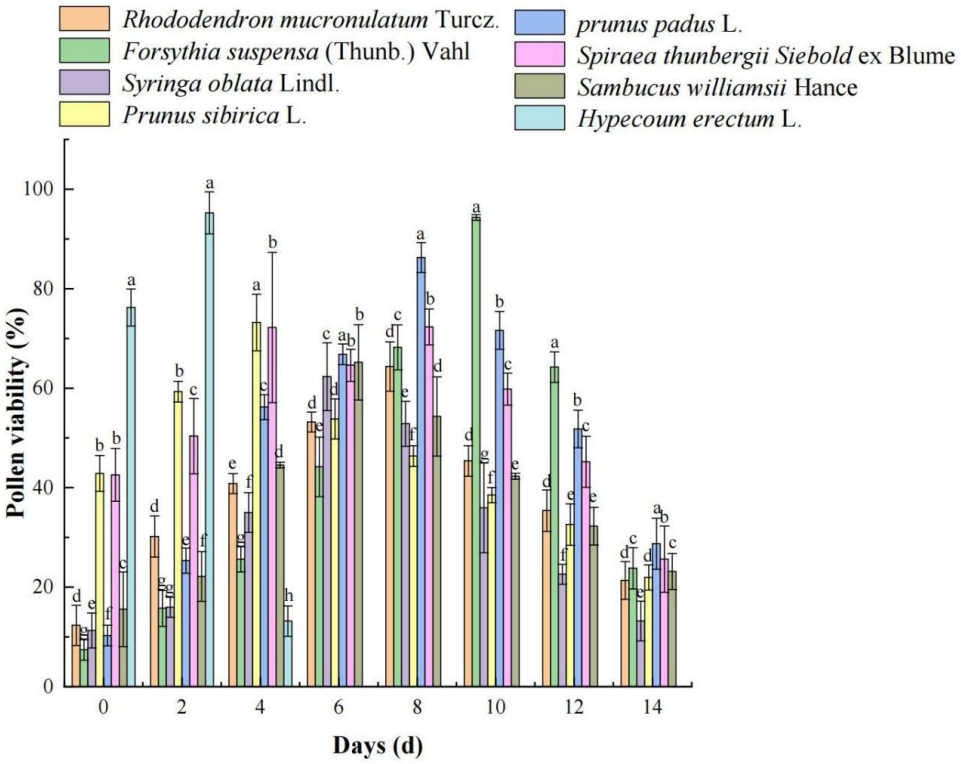


***Figure 5.*** *TTC staining method to detect pollen vitality. The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions*

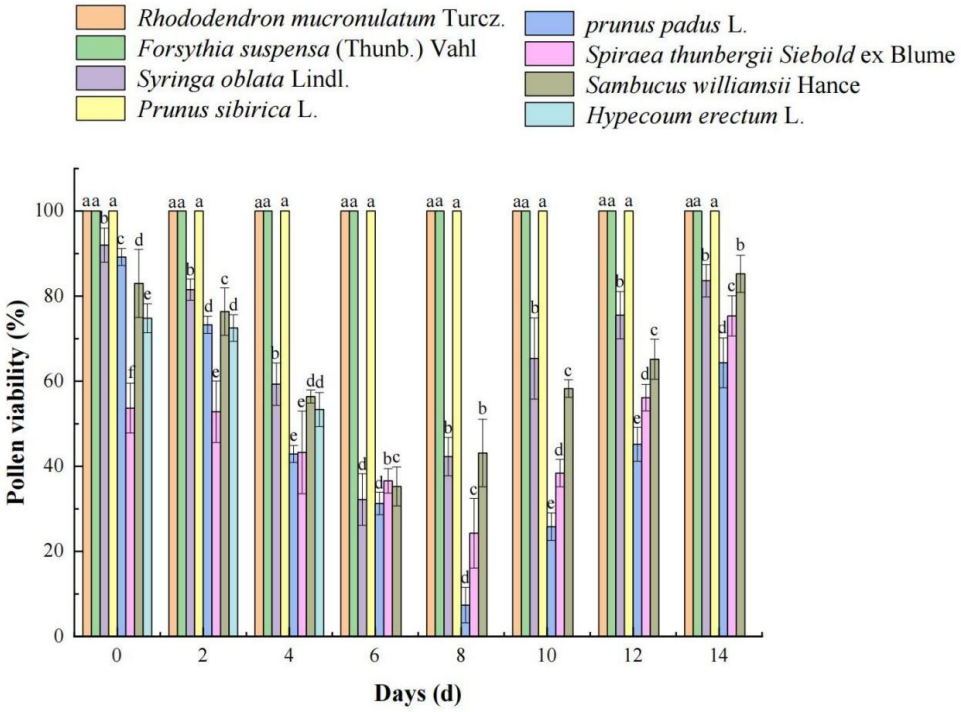


***Figure 6.*** *Red ink staining method to detect pollen vitality. The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions*


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*Determination of pollen viability by sucrose solution germination method*

The peak value of pollen viability of *H. erectum* was on the 0th d, and then the pollen viability decreased and reached 0 after the 6th d. There exists a significant difference between *F. suspensa* and *S. oblata* from the 0th d to the 4th d ($p < 0.05$), and an highly significant difference from the 6th d to the 14th d ($p < 0.01$). There are highly significant differences between *P. sibirica* and *S. thunbergii* at 0 d, 2 d, 6 d, and 14 d ($p < 0.01$). A significant difference exists between *S. williamsii* and *H. erectum* ($p < 0.05$). No significant difference exists between *S. williamsii* and *R. mucronulatum* ($p > 0.05$) (*Fig. 7*).

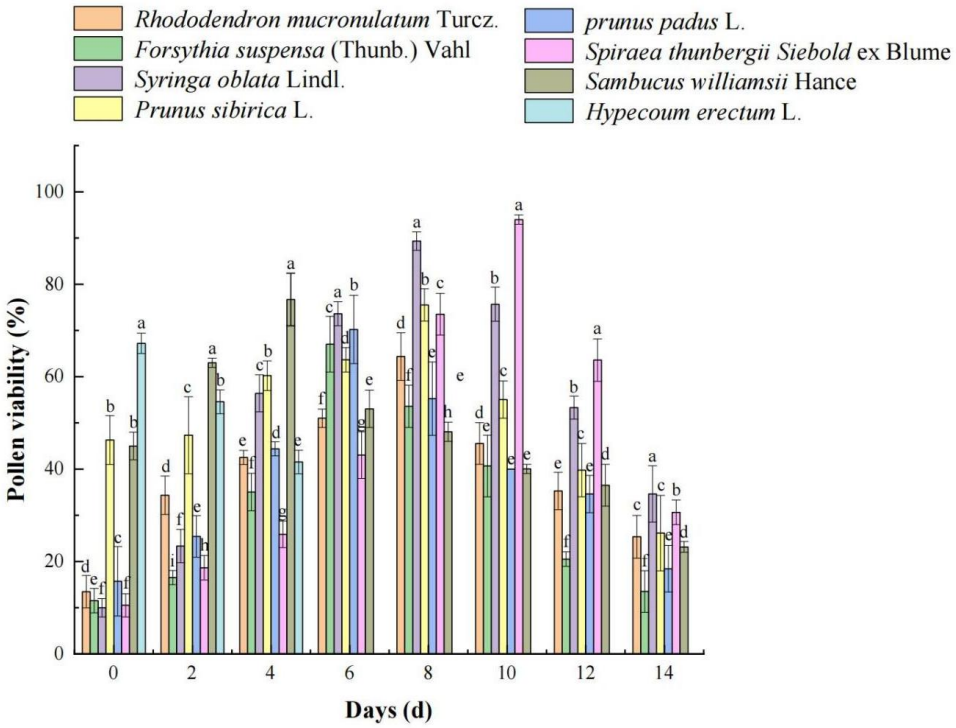


***Figure 7.*** *Pollen viability detection by sucrose solution germination method. The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions*

***Stigma receptivity***

During the 14 d of the study, except for *H. erectum* and *P. sibirica*, the stigma receptivity of the other 6 plants was strongest on the 8th d of flowering. The stigma receptivity of *P. sibirica* was strong on the 2nh d after flowering, but it decreased and eventually disappeared on the 6th d. The stigma receptivity of *H. erectum* was the highest on the 4th d after flowering, and began to decline on the 6th d, and there was no stigma receptivity after the 8th d (*Table 3*).

***Seed characteristics***

By measuring the seed setting rates of different types of plants, the results showed that the seed setting rates of the 8 plants were between 20% and 80% (*Eq. 3*). The seed setting rate of *P. padus* was the highest, while that of *H. erectum* was the lowest. There


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is a significant difference between *P. padus* and *P. sibirica* ($p < 0.05$), *R. mucronulatum* and *S. williamsii* exhibit a highly significant difference from *H. erectum* ($p > 0.01$), and there is no significant difference between *F. suspensa*, *S. oblata*, and *S. thunbergii* ($p > 0.05$) (*Fig. 8*).

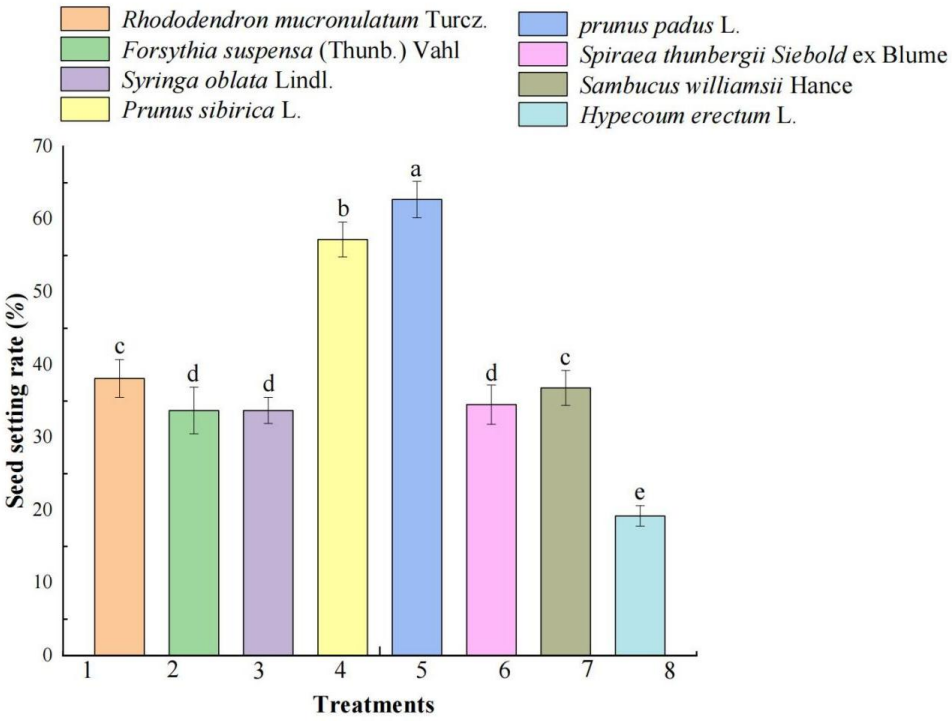


***Figure 8.*** *Seed setting rate of 8 early-spring plants. The different letters following the values indicate that there are significant differences ($p < 0.05$) or highly significant differences ($p < 0.01$) among the 8 plants under the same conditions*

## Discussion

### *Relationship between flowering phenology and plant reproductive characteristics*

Flowering phenology is one of the important life history characteristics of plants and an important part of plant reproductive ecology research. It mainly studies the relationship between plant flowering patterns and abiotic factors, as well as the genetic basis and natural selection of plant flowering (Ollerton and Lack, 1992). The reproductive activities of most plants tend to occur in spring because sufficient available water, suitable temperatures and abundant insects provide good conditions for the reproductive success of plants (Pías et al., 2001). The exploration of its adaptive significance can be expressed at multiple levels such as community family, genus, species, population, individual, inflorescence and single flower. The flowering phenology of plant individuals or groups can be quantified by a series of parameters such as flowering time and flowering duration, which can significantly affect the reproductive success of plants (Mclntosh, 2002). The flowering phenology observation of 8 early-spring plants showed that the flowering period of the 8 early-spring plants was relatively long, generally lasting from mid-march to mid-to-late June. The flowering time was long and there was obvious flowering asynchrony. This long-lasting


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and asynchronous flowering not only ensures that the plants can successfully complete the pollination and fertilization process over a longer period of time and thus ensure reproductive success, but also reduces the adverse effects of harsh natural environment during the flowering period, such as late spring cold, on their reproductive success. This is a reproductive strategy formed by the 8 early-spring plants as a result of their long-term adaptation to environmental influences.

***Table 3.*** *Results of the stigma pollination test of 8 plant species*

| Species | Flowering days (d) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0 d** | **2 d** | **4 d** | **6 d** | **8 d** | **10 d** | **12 d** | **14 d** |
| *Spi. Thu* | - | ++ | ++ | +++ | +++ | ++ | + | - |
| *Syr. Obl* | + | ++ | ++ | ++ | +++ | ++ | + | - |
| *For. Sus* | + | ++ | ++ | +++ | +++ | +++ | ++ | ++ |
| *Pru. Pad* | + | ++ | ++ | +++ | +++ | +++ | ++ | + |
| *Rho. Muc* | - | + | ++ | +++ | +++ | ++ | - | - |
| *Sam. Wil* | - | + | ++ | ++ | +++ | ++ | + | - |
| *Pru. Sib* | ++ | +++ | +++ | ++ | ++ | ++ | - | - |
| *Hyp. Ere* | + | ++ | +++ | - | - | - | - | - |

"+" represents there are a few bubbles, the stigma receptivity is weak; "++" represents there are more bubbles, the stigma receptivity is medium; "+++" represents there are a lot of bubbles, the stigma receptivity is strong; "-" represents there are no bubbles, the stigma receptivity is lost

### *Interaction between pollen viability, stigma receptivity and seed setting characteristics*

Pollen viability refers to the ability of pollen to survive, grow, germinate or develop. It is one of the reference indicators for whether breeding work can proceed smoothly. Pollen viability is directly related to the success rate of hybrid breeding (Sheng, 2007). Li et al. (2013) and Fan et al. (2012) used 3 methods to test the pollen vitality of *Cocos nucifera* L. and *Brassica* campestris, and found that the red ink staining method could not stain all the pollen grains of the plants, the TTC staining method had a higher measurement value, and the sucrose solution germination method could better distinguish between viable and aborted pollen. At present, the methods for determining plant pollen vitality include staining, in vitro germination assay, morphological assay, field pollination assay, etc. Among them, staining and in vitro germination assay are more commonly used (Yang et al., 2021). The staining method is simple and rapid, but it is greatly affected by the characteristics of pollen itself and cannot directly express the germination rate of pollen. The in vitro germination assay and field pollination assay are complicated and time-consuming, but the data of pollen germination assay is scientific and reliable (Wang et al., 2022). The combination of the TTC staining method and the sucrose solution germination method produced more accurate measurement results. This study also used these 3 methods to determine the pollen viability of 8 early-spring plants. The results showed that the red ink staining method could not stain *R. mucronulatum*, *F. suspensa*, and *P. sibirica*. The pollen viability of the 8 early-spring plants measured by the TTC staining method was higher than the value measured by the sucrose solution germination method. This indicates that the TTC staining method can be used to assist in the rapid detection of pollen viability, and the sucrose solution in vitro germination assay can be used to accurately determine the pollen viability of 8 early-spring plants.


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Stigma receptivity directly affects the success rate of plant sexual reproduction, and the duration of stigma receptivity varies and is closely related to species and varieties (Cai, 2023). The stigma receptivity of *F. suspensa* showed from the 2nd to the 14th d. Except for *H. erectum*, the stigma receptivity of the other 7 plants was strongest from the 6th to the 10th d, and the stigma receptivity of *H. erectum* disappeared from the 6th d. The benzidine-hydrogen peroxide method is generally considered to be a reliable method for detecting the receptivity of stigmas (Zhang et al., 2018). Xu et al. (2023) found that for *Dionaea muscipula* J. Ellis ex L., the stigma needs a longer time to be dyed brown instead of blue, but bubbles will still be produced. In this study, all 8 early-spring plants produced bubbles when reacted with the benzidine-hydrogen peroxide solution, which shows that the number of bubbles can be used to determine the strength of the plant's stigma vitality.

The research conducted by Liu et al. (2020) showed that the pollen activity of *Artemisia wudanica* Liou & W. Wang peaked on the 3rd to 4th d, and its stigma receptivity was also the strongest on the 3rd to 4th d, and pollen activity was positively correlated with stigma receptivity. In this study, except for *H. erectum*, the other 7 plants had the strongest pollen vitality and stigma receptivity on the 6th to 10th d. The pollen vitality of *H. erectum* disappeared after the 6th d, and the change of its stigma receptivity was consistent with this, indicating that the stronger the pollen vitality of the plant, the stronger the stigma receptivity. Cai et al.'s (2023) study showed that *Passiflora edulis* Sims setting rate was extremely significantly correlated with pollen viability and significantly correlated with stigma receptivity, indicating that both pollen viability and stigma receptivity have a significant impact on the seed setting rate. The higher the pollen viability and stigma receptivity of the plants in this study, the higher their seed setting rate, which is consistent with the research results.

### *Number of pollen grains and diversity of morphological characteristics of pollen grains*

The amount of pollen varies among different varieties of plants, which may depend on the species' inherent genetic traits (He et al., 2009). Among the comparisons of pollen numbers in 8 plant species, *S. oblata* and *P. sibirica* have the highest pollen numbers, making them more suitable as pollen donor plants. Therefore, the author hypothesizes that the number of pollen grains may be related to certain reproductive characteristics of the plant. Pollen morphological characteristics are an important basis for plant relationship, plant classification, and cultivar approval. Traditional morphological classification is greatly affected by the environment and its objectivity is limited to a certain extent. However, pollen morphological characteristics are controlled by genes, are less affected by external environmental conditions, and have strong genetic stability (Turaga et al., 2022). According to the observation results of pollen of 8 early-spring plants, the pollen of this genus showed a certain diversity in shape and size, the ratio of the length of the polar axis to the equatorial axis to the length of the germination pore, and the outer wall decoration. This study found that the pollen polar axis lengths of 8 early-spring plant species ranged from 17.44 to 54.9 μm. According to the research of Wei (2002), pollen with a polar axis length of 10-20 μm belongs to small-grain pollen, 25-50 μm belongs to medium-grain pollen, and 50-100 μm belongs to large-grain pollen. The subtle features of pollen grains, such as the germination organs and outer wall patterns, vary with plant species and remain stable in various plants (Wang et al., 2019). The types of pollen germination grooves all extend from the


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equator to the poles and no joint germination grooves occur at the poles (Zhang et al., 2023b). *R. mucronulatum* has diffuse pore germination grooves, and the other 7 plants have three-groove germination grooves. There are certain differences between the length of the germination groove and the width of the germination pore of the pollen of 8 plants, and the outer wall decoration of their pollen grains has multiple types of patterns. According to the current research conclusions on pollen micromorphology, pollen with different outer wall decorations may exist in the same family, genus, subgenus and species (Liu et al., 2022). Therefore, we speculate that the diversity of pollen grain morphology may provide a certain the theoretical basis for plant taxonomy.

## Conclusions

To sum up, flowering phenology runs through the entire growth process of the plant and expresses its flowering form and characteristics in a certain way. The pollen vitality of *R. mucronulatum*, *F. suspensa* and *P. sibirica* is not suitable for detection by red ink staining method. TTC staining method can be used as an auxiliary method to detect pollen vitality, and sucrose solution germination method can be used as the main method to accurately detect the pollen vitality of 8 plants. The differences in the number and morphology of pollen grains are related to their genetic characteristics, and the differences in pollen morphology may serve as a basis for plant classification. The higher the pollen viability and stigma receptivity of a plant, the greater its seed setting rate. In this study, pollen characteristics and flowering process of 8 different early-spring plants in the same area of northern China were observed and studied, and their differences were explored. This paper provides some reference for the study of flowering phenology, pollen and reproductive growth characteristics of plants in early spring.

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## REFERENCES

[1] Cai, M. L., Chang, X. L. (2007): Research on the identification method of Angelica dahurica. – Journal of Shaanxi University of Traditional Chinese Medicine 30(2): 56-57.
[2] Cai, Z. Y., Dong, L., Wang, H. C. (2023): Pollen viability and stigma pollinability at different developmental stages of passion fruit flowers and their effects on fruit set. – Journal of Fruit Tree 40(5): 969-977.
[3] Chen, S. P., Dai, X. Z., Guo, T. J. (2011): The phenological phase of major landscape trees in Changchun City and their application in planting design. – Journal of Jilin Agricultural University 33(2): 189-194.
[4] Diao, M. J. (1994): On *Prunus sibirica* L. resources and their rational development and utilization [J]. Natural Resources. – Natural Resourcesn 1994(2): 70-73.
[5] Fan, B. L., Zhang, Y. X., Wu, Z. Z. (2012): Comparison of pollen viability determination methods in different strains of rapeseed. – Anhui Agricultural Science 40(5): 2596-2597.
[6] Fang, X. L., Wang, Q. G. (2021): Research progress on the impact of increased nitrogen and precipitation changes on early spring plants under the background of global climate change. – World Ecology 10(4): 592-601.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_651669

[7] Gao, B., Han, X. Y., Tang, L. (2022): Prediction of flowering period of three early spring plants in Jilin city based on meteorological factors. – Journal of Beihua University (Natural Science Edition) 23(3): 311-314.
[8] Hao, C. Y., Liu, P., Guo, W. D. (2006): Caprifoliaceae part of plant DNA extraction method research. – Journal of Zhejiang Normal University: Natural Science Edition 29(1): 92-98.
[9] He, C. Y., Tan, X. F., Yuan, D. Y. (2009): Determination of pollen count and pollen germination rate of 7 oil tea species. – Journal of central south forestry university of science and technology 29(1): 5.
[10] Li, B., Chen, G. Y., Wei, G. D. (2023): Investigation on wild Roseaceae plant resources in Yantai, Shandong Province. – Chinese Wild Plant Resources 42(2): 103-109.
[11] Li, H. S., Wang, C. M., Wang, B. C. (2013): Study on pollen viability of several major coconut varieties in China. – Jiangxi Journal of Agriculture 25(9): 11-14.
[12] Li, S., Li, W., Yang, D. L. (2004): Determination of Pyrus pyrifolia pollen viability and its effect on fruit setting rate. – Journal of Horticultural Science and Technology 21(1): 3.
[13] Li, Z., Dong, R. (2021): Studies on pollen viability and stigma pollinability of five subspecies of wild pea. – Southern Agriculture 15(28): 16-19.
[14] Liang, L., Kong, D. Z., Liu, Z. X. (2022): Comparative analysis of methods for detecting lotus pollen viability. – Jiangsu Agricultural Science 50(2): 131-136.
[15] Liao, Q. F., Bi, K. S. (2004): Research progress on the chemical constituents and pharmacological activities of Sambucus species. – Chinese Journal of Chinese Materia Medica 29(2): 109.
[16] Liu, H., Cai, P., Wan, T. (2020): Study on the floral dynamics, pollen viability and stigma fertility of Artemisia ordosica. – Chinese Journal of Grassland 42(1): 5.
[17] Liu, L., Zhang, L. L., Cheng, G. L. (2022): Pollen morphology of 10 species of Primula spp. in the Sedera Mountains and their taxonomic significance. – Guangxi Plant 42(10): 1675-1683.
[18] Liu, Q. L., Zhou, G. L., Tian, G. (2005): Caprifoliaceae plant application in landscape in Xinjiang. – Journal of Xinjiang Agricultural Science 42 (B06): 84-86.
[19] Liu, Y., Lu, L. F., Wu, W. L. (2024): Research progress on seed dormancy characteristics and chemical treatment methods of Rosaceae fruit trees. – Fruit Trees of Southern China 53(1): 248-253.
[20] Liu, Y. N., Zhou, D., Liu, W. (2011): Effects of culture medium and storage conditions on pollen viability of plants in the genus Rhododendron. – Journal of Northeast Forestry University 39(12): 3.
[21] Lu, D., Li, P. Y., Li, J. H. (2003): Study on the Chemical components of lavender leaves. – Chinese Herbal Medicines DOI: CNKI:SUN:ZCYO.0.2003-08-006.
[22] Mcintosh, M. E. (2002): Flowering phenology and reproductive output in two sister species of Ferocactus (Cactaceae). – Plant Ecology 159(1): 1-13.
[23] Ollerton, J., Lack, J. (1992): Flowering phenology: an example of relaxation of natural selection. Trends. – Trends in Ecology and Evolution 7(8): 274-276.
[24] Pías, B., Guitián, P. (2001): Flowering phenology and pollen-to-ovule ratio in coastal dune communities near Eurosiberian-Mediterranean border in the NW Iberian Peninsula. – Flora 196(6):475-482.
[25] Sanzol, J., Rallo, P., María, H. (2003): Asynchronous development of stigmatic receptivity in the pear (Pyrus communis; Rosaceae) flower. – Journal of Botany 90. DOI: 10.3732/ajb.90.1.78.
[26] Shen, Y. Y., Shang, S. N., Liu, M. Q. (2006): Pollen morphology of Bilberry (Azaleaceae): A new method for measuring tetrazygous pollen using polar coordinates. – Wuhan Botanical Research 24(4): 4.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_651669

[27] Sheng, J. (2007): The relationship between pollen viability and cold tolerance of different self-pollinated varieties of Cucurbita pepo at low temperature. – Shandong Agricultural University.
[28] Su, Y. Y., Wang, K. C., Xue, Q. (2016): Study on pollen viability and stigma pollinability of patchouli from different origins. – Journal of Grass Industry 25(9): 189-196.
[29] Sun, Z. Y., Zhao, L. J. (2024): Research on the introduction and reproduction of Rhododendron indicum – The 70th Anniversary Conference of the Chinese Botanical Society.
[30] Tian, L. M., Cao, Y. F., Han, H. J. (2007): Cultivation Technology and application of Spiraea salicifolia in landscape. – North Gardening 6: 185-186.
[31] Tu, Y. H., Gao, N. N. (2007): Clover privet genera plants main chemical components and pharmacological activity research. – National Physician Characters 5: 212-214.
[32] Tulaga, T., Zhang, Z. Q. (2022): Comparison of pollen morphological characters of alfalfa varieties. – Northern Agricultural Journal 50(2): 94-102.
[33] Wang, B., Zhao, Y., Qiu, J. (2022): Study on the germination characteristics of Rosa multiflora pollen. – Western Forest Science 51(3):18-2339.
[34] Wang, H. M., Zhang, Q. Y., Zhang, C. (2019): Comparison of pollen viability determination and storage methods of different modern moonflower varieties. – Seed 38(3): 93-96.
[35] Wang, P. P., Tulson, G. T., Huang, J. H. (2016): Pollen morphology and its taxonomic significance of three plants of the genus Huperzia in Xinjiang. – Guangxi Plant 36(6): 663-673.
[36] Wei, Z. X., Jin, Q. J., Wang, H. (2002): Studies on the pollen morphology of Chionanthaceae and related taxa. – Journal of Plant Taxonomy and Resources 24(4): 483-496.
[37] Xiao, H. Y., Shen, B. R., Fan, G. R. (2024): Determination of repellent activity of extracts from Roseaceae against mosquitoes. – Southern Forestry Science 52(2): 48-53.
[38] Xu, F., Zhang, Y. P., Zhao, X. (2023): Studies on pollen viability and stigma pollinability in flytrap. – Jiangxi Journal of Agriculture 35(6): 75-81.
[39] Xu, L. L., Chen, Y. S., Zhang, S. Q. (2007): Research on the effects of ABA on clover plant antifreezing. – Modern Agricultural Science and Technology 15: 2.
[40] Yang, Q., Fu, Y., Chen, Y. Y. (2021): Correlation analysis of blueberry pollen viability with endogenous hormones and polyamines. – Economic Forest Research 39(3): 10-17.
[41] Yang, R. L., Wei, X. Z., Bi, R. C. (2003): Shanxi Huoshan poppy plant pollen morphology. – Journal of Guangxi Plants 23 (3): 4.
[42] Zhang, B., Qin, K., Huang, T. (2022): Study on pollen viability and stigma pollinability of Ningxia wolfberry. – Hubei Agricultural Science 61(19): 83-86.
[43] Zhang, C. B. (2005): Analysis of ecological vulnerability of plant community in urban green space in Changchun. – Urban Environment and Urban Ecology 18(6):37-39.
[44] Zhang, N. C., Li, D. D., Yu, W. N. (2023a): Flammability analysis of herbaceous plants in early spring. – Forest Fire Prevention 41(2): 20-24.
[45] Zhang, Q. F., Yin, X. Y., Jia, H. S. (2018): Study on pollen viability and stigma pollinability of seven species of Iris. – Western Forestry Science 47(4): 21-25.
[46] Zhang, S. J., Yang, X. X., Luo, J. (2023b): Morphological characteristics of the pollen and its taxonomic significance in the Sedzira Mountains of Tibet. – Plant Research 43(5): 741-755.
[47] Zhang, Y. F., Hu, C. Q., Yang, Y. (2016): Observations on pollen morphology of 8 species of persimmon. – Journal of Horticulture 43(6): 1167-1174.
[48] Zhao, J. H., Zhao, Y., Zhang, Q., Wang, Y. S., Fu, J. H., Zhou, G. F. (2020): Study on the Pollen Morphology and Viability of Six Species of Rhododendron genus. – Acta Agronomica Sinica 10(11): 78-84.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_651669

[49] Zhao, W. Q., Wu, Z. C., Xiao, J. (2019a): Studies on pollen viability and stigma pollinability of pen bamboo (Pseudosasa viridula). – Journal of Bamboo 3: 7.
[50] Zhao, X. M., Ge, Y. M., Ren, P. (2019b): Comparison of the growth status of Sargentodoxa cuneata and Sargentodoxa matsudana in Dalian area. – New Agriculture 1: 1.
[51] Zhu, J. J., Meng, X. Y., Wu, Y. (2005): Analysis of the chemical composition of Prunus cerasifera fruit, stem, leaf, bark and trunk essential oil. – Analytical Chemistry 33(11): 1615-1618.


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