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Study of 15 Varieties of Herbaceous Peony Pollen Submicroscopic Morphology and Phylogenetic Relationships

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Abstract: *Paeonia lactiflora* Pall. is widely used in medicine, garden applications, and as a potted ornamental. Cultivated varieties of paeonifloras suitable for cut flowers are urgently needed. In this study, the pollen morphology of *P. lactiflora* was studied and the characters of different varieties were compared, so as to provide reference for selecting suitable parents for new hybrid varieties. We examined the pollen morphology of 15 herbaceous peony varieties using scanning electron microscopy and analyzed the external pollen morphology and genetic relationship of the varieties. The pollen grains of the studied varieties were spheroidal or subspheroidal, bilaterally symmetrical monads, circular in polar view, and circular or elliptical in equatorial view. The exine of the pollen grains was observed as being relatively smooth under the light microscope, with the area around the equatorial axis having more lumina under the scanning electron microscope. The pollen grain exine sculpture was either reticular or pit type. The pollen apertures were tricolporate, arranged longitudinally, and equally spaced. The pollen grains were of two sizes: medium and small. The differences between the varieties were mainly reflected in the exine sculpture of the pollen. The closer the genetic relationship between the 15 peony varieties, the more subtle the differences in the exine sculpture. In the same cluster group, the morphological characteristics of herbaceous peony pollen were correlated with the shapes of flower and scale buds and the texture of the petals. However, the study identified no direct correlation with the cultivar type and flower color.

Keywords: *Paeonia lactiflora* Pall; pollen; exine sculpture; phylogenetic relationships

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1. Introduction

Pollen carries a large amount of genetic information, and the structure of pollen grains is determined by species genes. Individual pollen grains are not easily affected by the environment and have relatively stable characteristics [1–6]. Understanding the morphological characteristics of pollen is important for discussing the origin, evolution, classification, and kinship of seed plants [7,8]. Some researchers have used a scanning electron microscope (SEM) to observe the pollen morphological characteristics of ornamental plants and explain the relationship between their species or varieties [9,10]. Studies on the pollen morphology of *Paeonia lactiflora* or *Paeonia* species have also been reported [11,12].

Paeonia lactiflora Pall. is a species of herbaceous flowering plant in the family Paeoniaceae. It is widely used in medicine, garden applications, and as a potted ornamental. However, some characters (such as flower type, color, size, pedicel length, and upright stem) of the existing varieties are not in line with the application standards of fresh cut flowers, which restricts the production and sale of such flowers. Breeding suitable peony varieties is important for the production of fresh cut flowers. In this study, 15 varieties of peony were selected for submicroscopic morphology analysis. We used SEM to observe morphological characteristics of pollen from these 15 varieties. We then analyzed the relationship between the tested varieties based on the external morphological characteristics of

the plant and exine sculpture of the pollen. The aim of the study was to provide a reference for the selection of parents of new cut flower varieties in hybrid breeding.

2. Materials and Methods

2.1. Test Materials and Sampling

The test was conducted at the Horticulture Experimental Station of Shandong Agricultural University, the Experimental Center of the College of Horticulture Science and Engineering of Shandong Agricultural University, and the College of Life Science of Shandong Agricultural University. The test materials comprised 15 varieties of herbaceous peony. The sampled peony varieties are shown in Figure 1 and detailed in Table 1. During late April to early May 2020, plant material was collected between 10:00 and 11:00 a.m. on clear days. Complete anthers with mature, but not cracked, powder were collected with tweezers and placed into a penicillin bottle filled with glutaraldehyde fixing solution. Five milliliters of the solution was then slowly drawn using a 10 mL syringe, and then pumped back in slowly. This process was repeated two or three times to ensure full contact between the solution and the anthers, and the anthers were then allowed to sink in the fixative. The bottles were then refrigerated at 0–4 °C until use.

Figure 1. External morphology of *Paeonia lactiflora* flowers: (**1**), Xueyuanhonghua; (**2**), Yangfeichuyu; (**3**), Fenchijinyu; (**4**), Gaoganhong; (**5**), Bingshan; (**6**), Xuefeng; (**7**), Tianshanhongxing; (**8**), Qingtianlan; (**9**), Guifeichacui; (**10**), Dafugui; (**11**), Hongfeng; (**12**), Chifen; (**13**), Hongxiuqiu; (**14**), Dongjingnvlang; (**15**), Hongfushi.

2.2. Electron Microscope

The pollen was dehydrated stepwise with alcohol and then evenly glued onto a circular metal platform with conductive double-sided tape using tweezers. The pollen was gold-coated in an IB-5 ion sputtering apparatus. The coated samples were scanned from different angles to observe the individual, local, and group sculpture of pollen grains and photographed using a JSM-6610LV scanning electron microscope. Twenty pollen grains from each species were selected for measurements and observations of sculpture type, polar axis length, equatorial axis length, lumina diameter, and ridge width. The values were averaged and the minimum–maximum range was recorded [13]. The submicroscopic morphology of the pollen grains was described according to Punt et al. [7].

Table 1. Main morphological characteristics of the varieties of *Paeonia lactiflora* in the test.

Pollen Code	Cultivar Type	Bulbil Shape	Bud Shape	Flower Color	Petal Texture
1	Pavilion type	Brush-shaped	Inclined sharp-pointed peach-shaped	White	Papery
2	Crown type	Projectile-shaped	Sharp-pointed peach-shaped	White	Papery
3	Rose type	Bamboo-shaped	Inclined sharp-pointed peach-shaped	Pink	Leathery
4	Colorful-ball type	Projectile-shaped	Sharp-pointed peach-shaped	Red	Waxy
5	Crown type	Bamboo-shaped	Sharp-pointed peach-shaped	White	Papery
6	Crown type	Bamboo-shaped	Sharp-pointed peach-shaped	White	Papery
7	Rose type	Bamboo-shaped	Sharp-pointed peach-shaped	White	Waxy
8	Rose type	Brush-shaped	Sharp-pointed peach-shaped	Soft red	Papery
9	Crown type	Bamboo-shaped	Sharp-pointed peach-shaped	Pink	Papery
10	Rose type	Bamboo-shaped	Sharp-pointed peach-shaped	Red	Waxy
11	Colorful-ball type	Bamboo-shaped	Sharp-pointed peach-shaped	Red	Papery
12	Rose type	Brush-shaped	Sharp-pointed peach-shaped	Pink	Papery
13	Crown type	Bamboo-shaped	Sharp-pointed peach-shaped	Red	Waxy
14	Rose type	Bamboo-shaped	Sharp-pointed peach-shaped	Pink	Leathery
15	Colorful-ball type	Brush-shaped	Sharp-pointed peach-shaped	Burgundy	Waxy

1, Xueyuanhonghua; 2, Yangfeichuyu; 3, Fenchijinyu; 4, Gaoganhong; 5, Bingshan; 6, Xuefeng; 7, Tianshanhongxing; 8, Qingtianlan; 9, Guifeichacui; 10, Dafugui; 11, Hongfeng; 12, Chifen; 13, Hongxiuqiu; 14, Dongjingnvlang; 15, Hongfushi.

2.3. Data Analysis

Based on the data of six indexes (pollen polar axis length, equatorial axis length, ratio of polar axis length to equatorial axis length, perforation diameter, number of perforations per unit area, and ridge width), systematic cluster analysis in IBM SPSS Statistics 19.0 software was used to develop a dendrogram [14].

3. Results and Analysis

3.1. External Pollen Forms

According to Erdtman's NPC classification system [8], the pollen of the tested peony varieties was classified as N3P4C5. The pollen grains were of monad type, spheroidal or subspheroidal (P/E ranging from 0.91 to 1.03), and symmetrical, except for a few pollen grains with an irregular shape (Table 2 and Figure 1). The size of normally developed grains in the same cultivar was ambiguous, but their size among different varieties was pronounced. The pollen in all tested varieties was circular in polar view and circular or elliptical in equatorial view (Table 2). The varieties with elliptical pollen in equatorial view were: Xueyuanhonghua, Yangfeichuyu, Fenchijinyu, Gaoganhong, Bingshan, Tianshanhongxing, Qingtianlan, Guifeichacui, Dafugui, Dongjingnvlang, and Hongfushi. The varieties with circular pollen in equatorial view were Xuefeng, Hongfeng, Chifen, and Hongxiuqiu.

Table 2. Pollen grain characteristics of *Paeonia lactiflora* varieties.

Pollen Code	Length of Polar Axis (μm)	Length of Equatorial Axis (μm)	P/E	Perforation Number per 100 μm^2	Lumina Diameter (μm)	Ridge Width (μm)	D/W	Shape of Two Poles	Type of Exine Sculpture
1	23 (18.5–26.4)	25.3 (21.2–28.4)	0.91	140	0.60	0.41	1.46	circular and elliptical	reticular
2	24.14 (21.6–26)	26.3 (21.6–29.4)	0.92	145	0.60	0.51	1.18	circular and elliptical	pit
3	24.3 (22–27.2)	26.4 (23.8–28.6)	0.92	85	0.82	0.59	1.39	circular and elliptical	reticular
4	26.7 (22.4–29.6)	26.9 (21.6–30.2)	0.99	102	0.55	0.58	0.95	circular and elliptical	pit

Table 2. *Cont.*

Pollen Code	Length of Polar Axis (μm)	Length of Equatorial Axis (μm)	P/E	Perforation Number per 100 μm^2	Lumina Diameter (μm)	Ridge Width (μm)	D/W	Shape of Two Poles	Type of Exine Sculpture
5	27.2 (23–31.5)	27.8 (24.8–30.2)	0.98	140	0.57	0.49	1.20	circular and elliptical	pit
6	24.3 (20–28.2)	25.4 (23.6–26.8)	0.96	170	0.44	0.51	0.86	circular	pit
7	27 (21.6–31.2)	26.3 (21.6–29.2)	1.03	70	0.57	0.69	0.83	circular and elliptical	pit
8	25 (32.1–22.2)	25.6 (20.6–28.5)	0.98	130	0.56	0.50	1.12	circular and elliptical	pit
9	25.4 (20.8–29.6)	25.7 (19–30.8)	0.99	125	0.48	0.48	0.86	circular and elliptical	pit
10	28.3 (24.4–31.6)	27.8 (22.6–31.4)	1.02	100	0.50	0.61	0.82	circular and elliptical	small pit
11	22.62 (17.8–26)	23.9 (14.8–28.4)	0.95	145	0.35	0.36	0.97	circular	pit
12	25.7 (20.8–28.87)	26.6 (22–28.4)	0.97	125	0.55	0.45	1.22	circular	pit
13	25.2 (21.8–27.8)	25.3 (20.4–27.6)	1.00	125	0.48	0.57	0.84	circular	small pit
14	22.4 (17.8–25.8)	24.3 (17.6–29.6)	0.92	160	0.37	0.42	0.88	circular and elliptical	small pit
15	24.7 (19–29.2)	26.2 (23–29)	0.94	160	0.49	0.41	1.2	circular and elliptical	pit

1, Xueyuanhonghua; 2, Yangfeichuyu; 3, Fenchijinyu; 4, Gaoganhong; 5, Bingshan; 6, Xuefeng; 7, Tianshanhongxing; 8, Qingtianlan; 9, Guifeichacui; 10, Dafugui; 11, Hongfeng; 12, Chifen; 13, Hongxiuqiu; 14, Dongjingnvlang; 15, Hongfushi.

3.2. Surface Ornamentation Characteristics of Pollen

The exine of the pollen grains of the 15 peony varieties was smooth at light microscope, and with a clearly defined lumina near the equatorial axis at SEM. From the equatorial axis to the poles, the diameter of the lumina gradually decreased, and the shape of the lumina openings was roughly circular or subcircular. According to the quantitative index of peony pollen morphology established by Yuan and Wang [15], the ratio of lumina diameter to ridge width was used as a reference index for the pollen exine sculpture. The exine sculpture can be divided into three types: reticular, pit, and small pit. The reticular exine type was present in Xueyuanhonghua and Fenchijinyu, the small pit type was found in Dafugui, Hongxiuqiu, and Dongjingnvlang, and the pit sculpture was present in Yangfeichuyu, Gaoganhong, Bingshan, Xuefeng, Tianshanhongxing, Qingtianlan, Guifeichacui, Hongfeng, Chifen, and Hongfushi (Table 2 and Figure 2).

3.3. Apertures of the Pollen Grains

The pollen apertures of the 15 herbaceous peony varieties were tricolporate, arranged longitudinally, and equally spaced. The trenches demonstrated slight and gradual narrowing toward the poles along the polar axis. The width of the pores and colpi varied slightly between the varieties. The pore membrane had protrusions in Gaoganhong, Bingshan, and Xuefeng and was without protrusions in Xueyuanhonghua, Yangfeichuyu, Fenchijinyu, Tianshanhongxing, Qingtianlan, Guifeichacui, Dafugui, Hongfeng, Chifen, Hongxiuqiu, Dongjingnvlang, and Hongfushi (Figure 2).

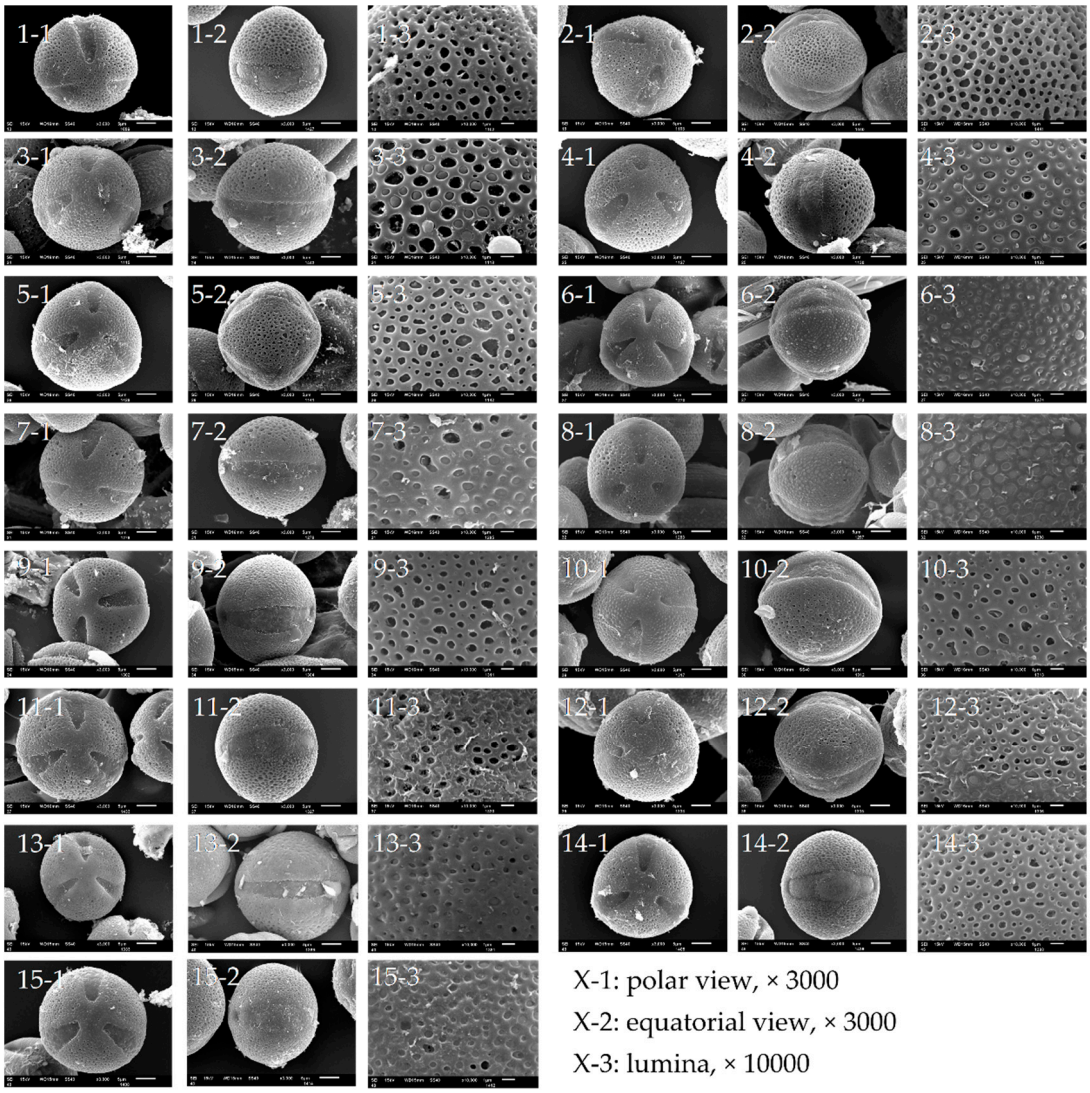

Figure 2. Pollen morphological characteristics of *Paeonia lactiflora* varieties: 1-1, 1-2, 1-3: Xueyuanhonghua; 2-1, 2-2, 2-3: Yangfeichuyu; 3-1, 3-2, 3-3: Fenchijinyu; 4-1, 4-2, 4-3: Gaoganhong; 5-1, 5-2, 5-3: Bingshan; 6-1, 6-2, 6-3: Xuefeng; 7-1, 7-2, 7-3: Tianshanhongxing; 8-1, 8-2, 8-3: Qingtianlan; 9-1, 9-2, 9-3: Guifeichacui; 10-1, 10-2, 10-3: Dafugui; 11-1, 11-2, 11-3: Hongfeng; 12-1, 12-2, 12-3: Chifen; 13-1, 13-2, 13-3: Hongxiuqiu; 14-1, 14-2, 14-3: Dongjingnvlang; 15-1, 15-2, 15-3: Hongfushi.

3.4. Pollen Grain Size

The pollen grains of the 15 peony varieties were divided into two size categories: medium (25–50 μm) and small (10–25 μm) (Table 2).

Seven varieties had a polar axis between 10 and 25 μm: Xueyuanhonghua, Yangfeichuyu, Fenchijinyu, Xuefeng, Hongfeng, Dongjingnvlang, and Hongfushi. Among them, the pollen grains of Hongfeng were the smallest (22.62 μm × 23.9 μm).

Eight varieties had a polar axis between 25 and 50 μm: Gaoganhong, Bingshan, Tianshanhongxing, Qingtianlan, Guifeichacui, Dafugui, Chifen, and Hongxiuqiu, with Dafugui having the largest pollen grains (28.3 μm × 27.8 μm).

3.5. Cluster Analysis

The systematic cluster analysis diagram of pollen morphology directly reflects the similarity of various units in the pollen exine sculpture (Figure 3). The closer the kinship, the earlier the aggregation. According to this, the species with close kinship can be determined, and the tested varieties can be divided into three groups.

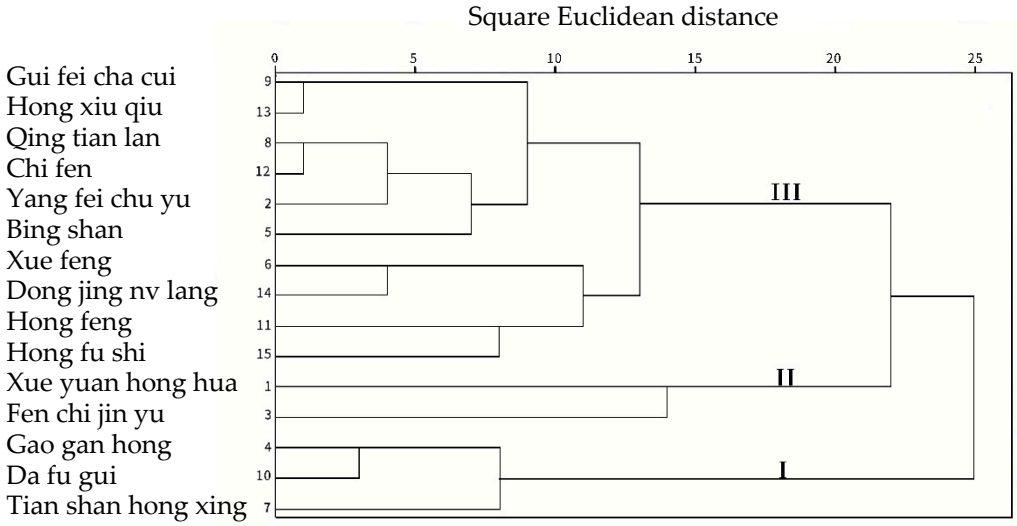

Figure 3. Clustering diagram of 15 varieties of *Paeonia lactiflora*.

The pollen morphology of the three varieties in Group I was nearly identical, and the length of polar axis values were mostly 26–28.5 μm. All of the pollen grain indexes of the two varieties in Group II were consistent, except for pore density, which varied slightly between the two varieties. Group III includes 10 varieties characterized by the lumina type of pollen pit, circular or subcircular pollen shape in polar view, and P/E values ranging from 0.9 to 1. The ridge width was 0.36 μm in Hongfeng and 0.45–0.55 μm in the other nine varieties.

4. Discussion

4.1. Similarities and Differences in Pollen Submicroscopic Morphological Characteristics among Herbaceous Peony Varieties

The pollen outer wall sculpture varies with species [16,17]. The SEM revealed both similarities and differences in the pollen grains among the 15 herbaceous peony varieties that we examined. The differences were mainly reflected in the external morphology, exine sculpture, apertures, and pollen grain size. In terms of the external morphology, the pollen grains were circular in the polar view, and circular to elliptical in the equatorial view. The equatorial view can be used as a basis for analyzing the relationships among the 15 varieties.

In this study, the pollen grain exine was relatively smooth under the light microscope, and the lumina near the equatorial axis was clear under the SEM. From the equator to the poles, the diameter of the lumina gradually decreased. The lumina shape was roughly circular or elliptical. The morphological differences in the pollen grains were mainly reflected in the number of perforations per unit area, lumina diameter, ridge width, shape in polar view, and sculpture type. The number of perforations per unit area varied with cultivar. There were three basic types of sculpture: reticular, pit, and small pit. The lumina diameters and ridge widths of individual grains of the same pollen were not equal, and the size difference varied with cultivar. There were some differences between the results of this study and those of Xi [18]. The exine sculpture, shape, and size of the pollen grains among different individuals of the same species are not affected by planting location, showing strong conservation [19]. Different test materials may lead to the differences in the above conclusions.

The pollen grains in the studied varieties were tricolporate, arranged longitudinally, and equally spaced. The trenches gradually and slightly narrowed toward the poles along the polar axis. The spindle-shaped colpium was situated near the poles. The width of the colpi varied slightly among cultivar varieties. The pore membrane was either with or without protrusions. The varieties with protrusions included Gaoganhong, Bingshan, and

Xuefeng, while the other 12 varieties had no protrusions in the pore membrane. The size of the pollen grains varied by cultivar and were separated into medium (25–50 μm) and small (10–25 μm) sizes. The microstructure of the exine sculpture of the pollen grains was consistent among individuals of the same cultivar, but varied among different varieties. The results of this experiment are consistent with those reported by Jin et al. [20].

4.2. Kinship

Cross breeding is an important way to breed new cut flower varieties and is one of the most widely used and effective methods for breeding [21]. Therefore, understanding the morphological relationship between varieties can help to predict the values for target phenotypes when cross-breeding new varieties. According to the pollen morphological characteristics and cluster analysis diagram, the closer the genetic relationship between varieties, the more subtle the differences in pollen morphological characteristics and exine sculpture. This is consistent with the results of Hao and Ma [12]. The cluster dendrogram based on pollen morphology resolved the three varieties of Gaoganhong, Tianshanhongxing, and Dafugui in the same cluster group (Cluster I) and are therefore closely related. Their petals are waxy in texture, their scales and buds are bamboo shoot-shaped, and their flower buds are pointed peach-shaped. The varieties Xueyuanhonghua and Fenchijinyu belong to the same cluster group (Cluster II) and have the closest relationship. Their petals have a papery texture, the scales and buds are brush-shaped, and the flower buds are crooked peach-shaped. The remaining 10 varieties (Cluster III) were closely related on the basis of pollen morphology. They are characterized by pointed peach-shaped flower buds. The petals of four varieties, Xuefeng, Hongfeng, Dongjingnvlang, and Hongfushi, are leathery in texture, and their scales and buds are in the shape of bamboo shoots. The petals of the remaining six varieties are papery in texture, and the scales and buds are projectile-shaped. This indicated that the pollen morphological characteristics in Clusters I and II were consistent with the texture of the petals, the shape of the scale buds, and the shape of the flower buds. The morphological characteristics of pollen in Cluster III were consistent with the shape of the flower buds, but not with the texture of the petals or the shape of the scale buds.

5. Conclusions

The aim of this study was to provide a basis for the selection of parents of new cut flower varieties in hybrid breeding. The 15 tested peony varieties were clustered into three groups. The pollen morphological characteristics correlated to a certain degree with petal texture and the shape of the scales and flower buds. There was no direct correlation between the morphological characteristics of the pollen and the cultivation type or flower color. These phenotypes may be controlled by multiple genotypes or are the results of long-term natural and artificial selection, which warrants further study. In this study, we studied the pollen morphology of *P. lactiflora* and compared the characteristics of different varieties. Future research will examine the molecular biology, plant morphology, and plant physiology of the tested varieties to provide a reference for the breeding of *Paeonia* cut flowers.

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