

## Observation of Pollen Grains of Ten Hydrangea varieties with Scanning Electron Microscope (SEM)

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**Abstract** In order to analysis the difference in pollen morphological characteristics of the hydrangea varieties and pollen fertility, scanning electron microscope was used to observe the pollen characteristics of 10 varieties of hydrangea, parameters of pollen grains such as shape, the ratio of long and short axes, germination groove, ornamentation and polar surface characteristics. In Observe the characteristics of pollen grain shape, aspect ratio, germination groove, ornamentation, polar surface and other characteristics of the 10 *Hydrangea macrophylla* through electron microscope scanning to analyze the differences between different Hydrangea varieties and pregnancy pollen grains sex. The results indicated that the pollen grains of the fertile flowers of 10 Hydrangea are all long spherical, except for the sterile flowers of 'Endless summer' and 'Otaksa', all the others are long spheroids. The all have with three germinating grooves, which are long splitting into to the germination grooves of the poles. The pollen belonging to the N3P4C5 type pollen, the polar face shapes of pollen grains were different in and the outer wall decoration is hole-shaped. But only the 'Endless summer' and 'Otaksa' infertility flowers were super-long spheres. The polar faces of pollen grains have different shapes, such as 'Endless Summer', 'Otaksa', 'Mona Lisa', 'Head Flower' and 'First Love' are trilobate circulares, others are blunt triangles. The length and ridge width of germination groove of different varieties were also different, and only the different pregnant pollen grains of 'Maman rose' had significant differences. The size, shape and distribution of the pores are different, but the morphological characteristics of different fertile pollen grains of the same variety are not different. In conclusion, the external walls decoration of pollen grains can be used as one of the basis for the identification of Hydrangea varieties, but not for the identification of pollen fertility. The length of the germination groove of different varieties of pollen grains differs from the width of the ridge surface of the germination groove, as well as the size, shape and distribution characteristics of the holes. However, there is no significant difference in the morphological characteristics of the outer wall decoration patterns of different pregnancy pollen grains of the same variety.

**Keywords** *Hydrangea macrophylla*; Pollen morphology; SEM; Cultivar identification

*Hydrangea macrophylla* is a deciduous shrub of *Hydrangea* genus in the family of Saxifragaceae, which is also known as Xiuqiuhua and Ziyanghua in Chinese. The inflorescence of *Hydrangea macrophylla* is large and colorful, which is of ornamental value. It can be used as a bouquet of flowers or dried flowers decoration. It is also an excellent green tree species. In recent years, it has been introduced and cultivated in large numbers in China, and has gradually become a "popular" plant, which is used in all kinds of theme flower shows.

At present, the research of *Hydrangea macrophylla* mainly focuses on *in vitro* culture, chemical composition separation and identification, cultivation management and so on (Ren, 2018, Journal of Green Science and Technology, 56(13): 165-167). However, there are few reports on the pollen morphological characteristics of *Hydrangea* varieties. It is recorded that there are more than 600 *Hydrangea* varieties, and many hybrids. It is difficult to verify their parents after multiple generations of reproduction, and even the classification of some *Hydrangea* varieties (such as *Hydrangea paniculata* 'Grandiflora' and *Hydrangea serrata* Ser.) has been controversial, resulting in confusion in plant naming and defects in the classification system (Zeng et al., 2018).

The palynology study on the interspecific relationship of Hydrangeoideae indicated that the morphological traits of pollen, the ornamentation of pollen outer wall surface and secondary ornamentation of Hydrangeoideae varieties had great reference value for species classification, and the study on pollen outer wall hierarchical structure and germination pores was helpful to further understand the evolution of pollen traits and intrageneric grouping of this subfamily (Chen, 1954; Hao and Hu, 1996; Zhang et al., 2019). Studies on pollen morphology of *Hydrangea* genus and its related genera showed that the taxonomic value of pollen morphology of *Hydrangea* genus and its related genera is mainly reflected in the definition of species (Zhang et al., 2019). Therefore, in this study, ten *Hydrangea* varieties were used as materials to observe the differences in morphological characteristics of pollen grains of different varieties and different fertility with scanning electron microscope (SEM), so as to further clarify the significance of morphological characteristics of pollen grains of *Hydrangea macrophylla* in taxonomy and provide scientific basis for the study of genetic diversity of *Hydrangea macrophylla*.

## 1 Results and Analysis

### 1.1 Population characteristics of pollen grains of ten *Hydrangea* varieties

The results of SEM (Figure 1; Figure 2; Figure 3) showed the number of pollen grains of *Hydrangea macrophylla* is single, and the number of pollen grains of different *Hydrangea* varieties is different. Pollen grains could be observed in all varieties of fertile flowers, but the sterile pollen grains of varieties 'Preziosa', 'Mona Lisa', 'Headdress flower' and 'Komachi' cannot be observed, which may be related to the small sterile buds of varieties. The pollen grains of fertile flowers of 'Komachi' were very few. The pollen grains of 'Preziosa', 'Headdress flower', and 'Porpcorn' were also relatively small, which may be related to flower type.

In the field of vision, there were different number of abnormal pollen grains in different varieties of *Hydrangea macrophylla*. The number of abnormal pollen grains in fertile flowers 'Maman rose', 'Endless Summer', and 'Mona Lisa' were the largest, which may be related to the small anthers and less nutrients for their growth and development. Abnormal pollen grains in the field of vision showed the irregular shape of shriveled and wrinkled, or the germination ditch was deeply depressed downward, which was the phenomenon of pollen degradation or pollen abortion, and may be one of the reasons for male sterility of *Hydrangea macrophylla*.

The normal pollen morphology of ten *Hydrangea* varieties has the following common characteristics: the appearance is prolate, and the equatorial plane has midday germination ditch. However, the size of pollen grains, germination ditch depth and ridge shape of different varieties were different.

### 1.2 Analysis of pollen characteristics of fertile flowers of ten *Hydrangea* varieties

#### 1.2.1 Morphological characteristics of pollen grains of ten fertile varieties

The pollen appearance of *Hydrangea macrophylla* is prolate, and isopolar, radially symmetrical, with equatorial three-hole ditch, and are midday long groove, groove extends to the two extremes do not converge on the polar plane. Pollen outer wall have hole pattern, according to NPC classification system, it belongs to N3P4C5 type pollen. Based on the L/W ratio, the pollen grains of the tested varieties were prolate and the pollen grains of 'Porpcorn' and 'Otaksa' were perprolate, which were significantly different from other varieties.

There were significant differences in pollen grain size and morphology among varieties (Table 1). The pollen grains of *Hydrangea macrophylla* belong to small pollen grains, with length of 16.10~25.50  $\mu\text{m}$  and an average of 17.34~21.74  $\mu\text{m}$ ; The width was 7.94~16.90  $\mu\text{m}$ , with an average of 9.61~12.05  $\mu\text{m}$ . Pollen size was expressed by the length of pollen. The order of pollen grains was as follows: 'Maman rose' > 'Porpcorn' > 'Headdress flower' > 'First love' > 'Lanarth' > 'Otaksa' > 'Mona Lisa' > 'Endless Summer' > 'Preziosa' > 'Komachi'. The pollen grains of 'Maman rose' and 'Porpcorn' were larger than other 8 varieties, and the pollen grains of 'Komachi' and 'Preziosa' were the smallest, and there were significant differences with other varieties. And there were no significant differences among the other six varieties. The uniformity of pollen grains was expressed by the amplitude of length change. The pollen grains of *hydrangea serrata* 'Preziosa' were the most uniform, and the variation of pollen grain size of 'Maman rose' was the largest among individuals.

The polar shapes of pollen grains were different from each other (Figure 2). The polar shapes of 'Endless Summer', 'Otaksa', 'Mona Lisa', 'Headdress flower' and 'First love' were three-lobed round, while the other varieties were blunt triangle.

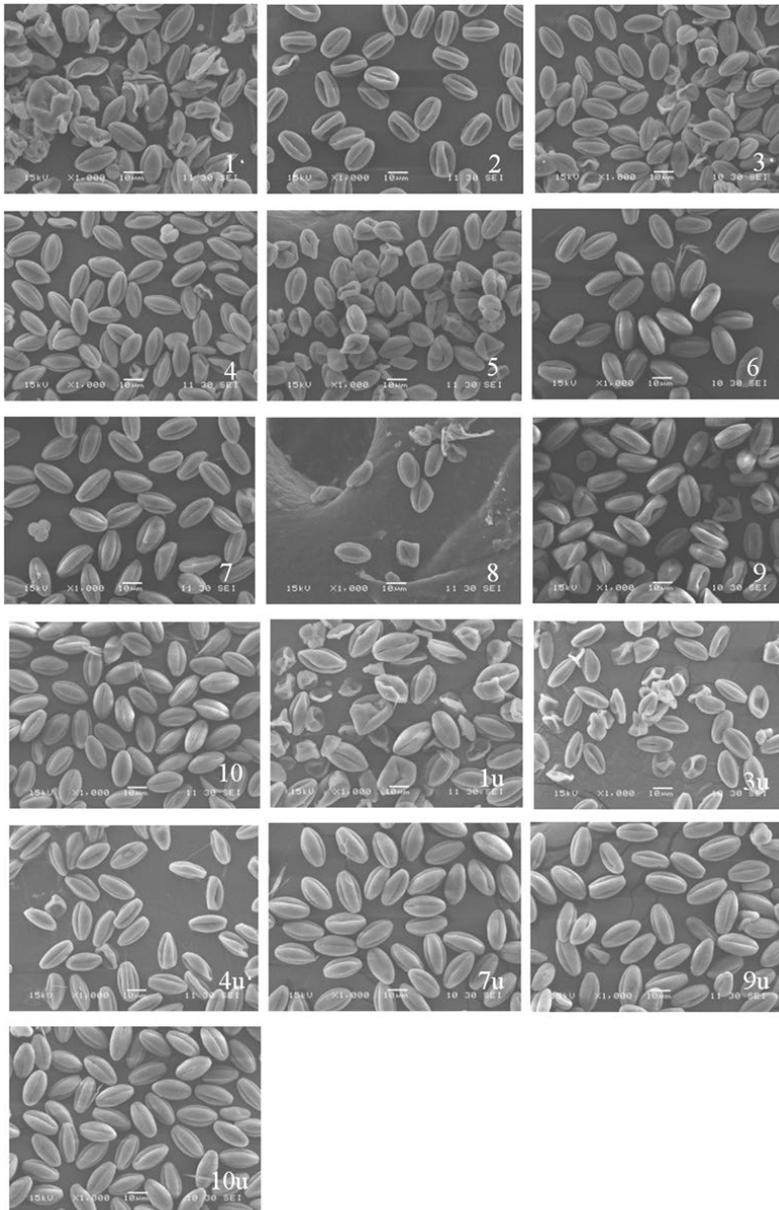


Figure 1 SEM micrographs of pollen grains population morphology

Note: Sexual male flower: 1: 'Maman rose'; 2: *Hydrangea serrata* 'Preziosa'; 3: 'Endless Summer'; 4: 'Otaksa'; 5: 'Mona Lisa'; 6: 'Headdress flower'; 7: 'Porpcorn'; 8: 'Komachi'; 9: 'Lanarth'; 10: 'First love'; Unisexual male flower: 1u: 'Maman rose'; 3u: 'Endless Summer'; 4u: 'Otaksa'; 7u: 'Porpcorn'; 9u: 'Lanarth'; 10u: 'First love'

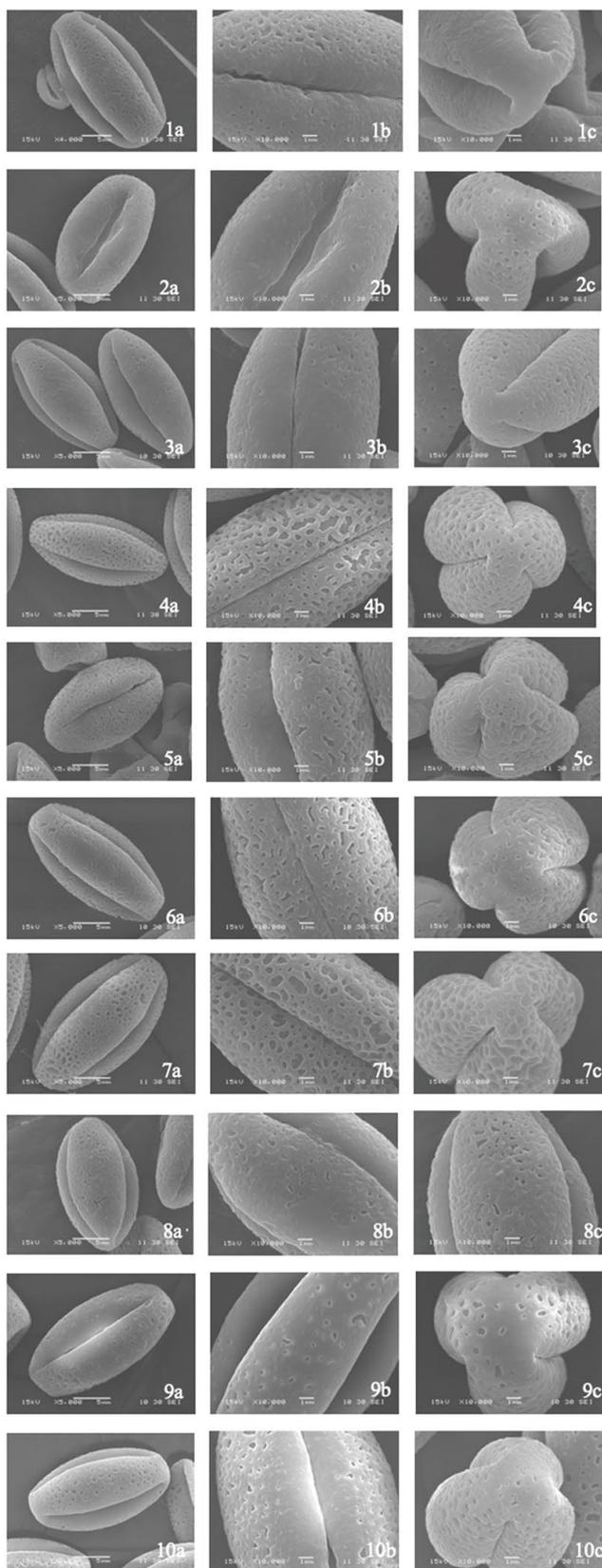


Figure 2 SEM micrographs of fertile flower pollen grains

Note: 1: 'Maman rose'; 2: Hydrangea serrata 'Preziosa'; 3: 'Endless Summer'; 4'Otaksa'; 5: 'Mona Lisa'; 6: 'Headdress flower'; 7: 'Porpcorn'; 8: 'Komachi'; 9: 'Lanarth'; 10: 'First love'; a: Pollen grain equatorial view; b: Pollen ornament; c: Polar view of pollen grains

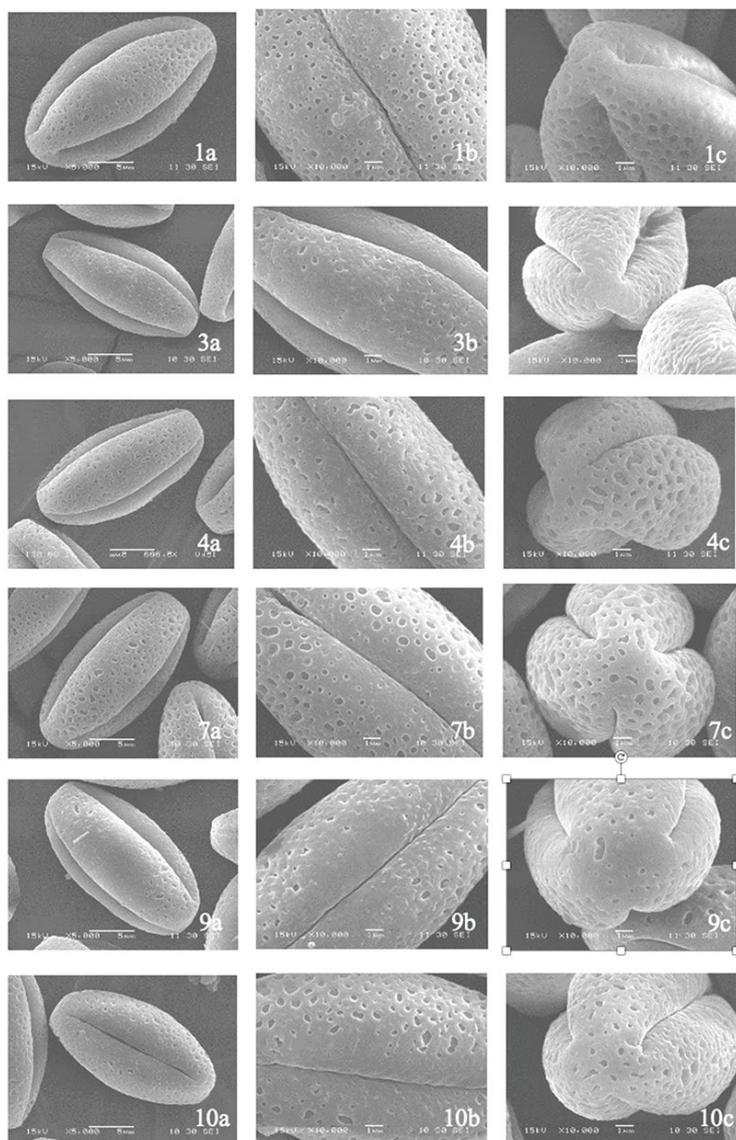


Figure 3 SEM micrographs of infertile flower pollen grains

Note: 1: 'Maman rose'; 3: 'Endless Summer'; 4: 'Otaksa'; 7: 'Porpcorn'; 9: 'Lanarth'; 10: 'First love'; a: Pollen grain equatorial view; b: Pollen ornament; c: Polar view of pollen grains

Table1 Comparison of pollen morphological characteristics of fertile flowers in ten *Hydrangea* varieties

Codes	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	L/W	Pollen shapes	Polar shape
1	21.74 $\pm$ 1.84 (19.20~25.50)a	12.05 $\pm$ 1.62 (9.62~15.30)a	1.82 bcd	Prolate	Blunt triangle
2	17.73 $\pm$ 2.19 (11.70~19.40)d	11.20 $\pm$ 0.92 (10.10~13.20)ab	1.59 e	Prolate	Blunt triangle
3	18.30 $\pm$ 0.70 (17.40~19.70)cd	9.61 $\pm$ 0.40 (9.00~10.30)d	1.91 abcd	Prolate	Three-lobed round
4	19.37 $\pm$ 0.69 (18.20~20.40)bc	9.79 $\pm$ 0.54 (9.06~11.00)cd	1.98 ab	Prolate	Three-lobed round
5	18.48 $\pm$ 0.64 (17.40~19.90)cd	10.59 $\pm$ 0.70 (9.12~11.60)bc	1.75 d	Prolate	Three-lobed round
6	20.46 $\pm$ 0.98 (19.30~22.60)b	10.50 $\pm$ 0.55 (9.65~11.20)bcd	1.95 abc	Prolate	Three-lobed round
7	21.71 $\pm$ 0.96 (20.40~23.20)a	11.01 $\pm$ 1.15 (9.33~12.70)b	1.99 a	Prolate	Blunt triangle
8	17.34 $\pm$ 0.94 (16.10~18.60)d	9.90 $\pm$ 1.68 (7.94~11.60)cd	1.79 cd	Prolate	-
9	19.84 $\pm$ 1.39 (17.70~22.00)b	10.28 $\pm$ 0.64 (9.20~11.30)bcd	1.93 abc	Prolate	Blunt triangle
10	20.36 $\pm$ 0.46 (19.80~21.10)b	10.99 $\pm$ 0.64 (10.20~12.20)b	1.86 abcd	Prolate	Three-lobed round

Note: Lowercases are significant tested by Duncan at 0.05 level, No polar surface of 'Komachi'

### 1.2.2 Pollen germination groove characteristics of ten fertile *Hydrangea* varieties

Pollen grains of ten *Hydrangea* varieties have 3 germination grooves, parallel to the equator and equidistant distribution. The grooves are long splitting into to the germination grooves of the poles., and no furrows are formed at polar face. The germination groove length and germination groove width of the tested varieties were significantly different (Table 2). The pollen germination groove of ‘Maman rose’ was the longest, reaching 25.60  $\mu\text{m}$ , while the ‘Preziosa’ and ‘Komachi’ were the shortest, which were significantly different from other varieties. But there was no significant difference between ‘Preziosa’, ‘Endless summer’, ‘Otaksa’, ‘Mona Lisa’, ‘Komachi’ and ‘First love’. The germination groove width of ‘Maman rose’ were the widest, nearly twice as wide as ‘Preziosa’, which were different from other varieties. While there were no significant differences between ‘Endless summer’, ‘Headdress flower’, ‘Porpcorn’, ‘Komachi’ and ‘Lanarth’.

It could be observed that there were differences in morphological characteristics of germination groove type (Figure 2). The germination groove of hydrangea serrata ‘Preziosa’ was the widest, grooved and deep inward. The germination grooves of the other nine varieties were slit-shaped and deep inward, in which ‘Maman rose’, ‘Otaksa’, ‘Mona Lisa’ and ‘Porpcorn’ extending to the middle of the pole.

Table 2 Comparison of pollen germination ditch of ten *Hydrangea* varieties

Codes	Germination groove length ( $\mu\text{m}$ )	Germination groove width ( $\mu\text{m}$ )	Germination groove type
1	25.60±0.35a	8.31±0.64a	Equatorial three-hole ditch
2	18.97±0.76d	4.89±0.32d	Equatorial three-hole ditch
3	19.53±0.55cd	6.33±0.46bc	Equatorial three-hole ditch
4	19.80±0.26cd	5.31±0.32d	Equatorial three-hole ditch
5	19.17±0.84d	7.06±0.84b	Equatorial three-hole ditch
6	21.73±0.78b	6.81±0.32bc	Equatorial three-hole ditch
7	22.20±1.08b	6.93±0.53bc	Equatorial three-hole ditch
8	18.83±0.32d	7.02±0.11bc	Equatorial three-hole ditch
9	20.57±0.59c	6.56±0.15bc	Equatorial three-hole ditch
10	19.87±0.31cd	6.20±0.17c	Equatorial three-hole ditch

Note: Lowercases are significant tested by Duncan at 0.05 level

### 1.2.3 Pollen pattern features of ten fertile *Hydrangea* varieties

The outer wall decoration of pollen grains of ten *Hydrangea* varieties were hole pattern, but the detail patterns of outer wall decoration between different varieties were different, which showed that the perforation distribution density, size and shape were different (Table 3; Figure 2).

From the point of view of the density of the perforation distribution, ‘Maman rose’, ‘Otaksa’, ‘Mona Lisa’, ‘Headdress flower’ and ‘Komachi’ have more holes and closely arranged, while ‘Lanarth’ has the least holes and the sparsest distribution.

Different varieties of pollen outer wall decoration also have differences. Among them, the pollen grain outer wall of ‘Maman rose’, ‘Preziosa’, ‘Endless summer’ and ‘Lanarth’ was smooth, while ‘Mona Lisa’ and ‘Porpcorn’ were wrinkled. According to the different pollen grain perforation shapes, the tested varieties were divided into three categories: (1) Hole pattern, round eyelet: ‘Maman rose’, hydrangea serrata ‘Preziosa’, ‘Endless summer’, ‘Porpcorn’, ‘Lanarth’ and ‘First love’; (2) Hole pattern, thick stripe eyelets: ‘Mona Lisa’ and ‘Headdress flower’; (3) Hole pattern, irregular eyelet: ‘Otaksa’ and ‘Komachi’.

## 1.3 Analysis of pollen characteristics of infertile flowers of six *Hydrangea* varieties

### 1.3.1 Pollen morphological characteristics of infertile flowers of six *Hydrangea* varieties

The results of SEM showed that only six *Hydrangea* varieties pollen grains could be observed. The pollen morphology of infertile flowers of six varieties was prolate or ultra-prolate, and the germination groove was equatorial three-hole ditch. There were also significant differences in pollen grain size (represented by length) and morphology of infertile flowers (Table 4). ‘Maman rose’ was the largest, ‘Endless summer’ was the smallest,

which was consistent with the results of fertile flowers. According to the analysis of pollen grain shape by L/W value, it was found that 'Endless summer' and 'Otaksa' were ultra-prolate, and the others were prolate, while the L/W value of 'Maman rose' was the smallest, which was significantly different from that of other varieties.

Table 3 Comparison of pollen pattern features of ten *Hydrangea* varieties

Codes	Decorative features	Perforation distribution	
		Extreme-central-extreme	Polar face
1	Hole pattern, round eyelet	Smooth, eyelet: small-big-small-big-small, tightly distributed	Smooth, non-perforated
2	Hole pattern, round eyelet	Smooth, small perforations, sparse distribution	Wrinkled, small perforations
3	Hole pattern, round eyelet	Smooth, eyelet: small-none-small, sparsely distributed	Wrinkled, non-perforated
4	Hole pattern, irregular eyelet	Wrinkle-shaped, eyelet: big-small-big, sparsely distributed	Wrinkled, small perforations
5	Hole pattern, thick stripe eyelets	Wrinkle-shaped, with different perforations and uniform distribution	Wrinkled, None-perforations
6	Hole pattern, thick stripe eyelets	Wrinkled, eyelet: big-none-big, tightly distributed	Wrinkled, small perforations
7	Hole pattern, round eyelet	Wrinkle-shaped, eyelet: big-small-big, tightly distributed	Wrinkled, big perforations
8	Hole pattern, irregular eyelet	Wrinkle-shaped, eyelet: big-small-big, sparsely distributed	Wrinkled, small perforations
9	Hole pattern, round eyelet	Smooth, eyelet: big-small-big, sparsely distributed	Smooth, big perforated
10	Hole pattern, round eyelet	Wrinkle-shaped, eyelet: small-big-small tightly distributed	Smooth, big perforated

Table 4 Comparison of pollen morphological characteristics of infertile flowers of six *Hydrangea* varieties

Code	L (μm)	W (μm)	L/W	Germination groove length (μm)	Germination groove width (μm)	Pollen shape
1	22.61±1.26a	12.45±0.55a	1.82 b	23.17±0.32a	7.20±0.16a	Prolate
3	18.66±1.02d	9.32±0.69c	2.01 a	19.70±0.62d	6.19±0.50b	Ultra prolate
4	20.04±0.46bc	10.01±0.90b	2.02 a	20.10±0.35cd	6.66±0.32ab	Ultra prolate
7	20.53±0.91bc	10.59±0.82b	1.95 a	21.17±1.37bc	7.13±0.38a	Prolate
9	20.72±1.05b	10.42±0.68b	1.99 a	21.70±0.56b	7.16±0.09a	Prolate
10	19.69±0.67c	10.36±0.33b	1.90 ab	20.67±0.31bcd	6.62±0.09ab	Prolate

It could be observed that there were differences in the outer wall morphology, perforation shape and density, and germination groove characteristics of pollen grains among the six infertile varieties (Table 5; Figure 3). In which, the perforation of 'Otaksa' was irregular, which was significantly different from other varieties. The characteristics of pollen germination groove of 6 infertile varieties were consistent with that of fertile flowers.

### 1.3.2 Comparison of the pattern features of fertile and infertile flowers on the six *Hydrangea* varieties

Among the six *Hydrangea* varieties, the pollen grains of infertile flowers 'Endless summer' and 'Otaksa' were ultra-prolate, which were different from those of fertile flowers. Analyzed the comparison of the length and width of germination groove, we found that there were significant differences between different fertile flowers of 'Maman rose'. The outer wall decoration shape and distribution density of the perforation of the same variety were similar, but the polar characteristics were different. Fertile flowers of 'Maman rose' have no holes on the extreme surface, but infertile flowers have larger holes, with the large number is and close, and other varieties have no significant differences, indicating that the morphological characteristics of pollen grains of the same variety have little correlation with fertility.

Table 5 Comparison of the pattern features of infertile flowers on the six *Hydrangea* varieties

Codes	Perforation shape	Smoothness (extreme-central-extreme)	between holes	Distribution perforations	of Polar features
1	Round eyelet	Wrinkle-shaped, eyelet: big-small-big		Tightly	Smooth, small perforations, Dense
3	Round eyelet	Wrinkle-shaped, eyelet: small-big-small		Sparsely	Wrinkled, none-perforations
4	Irregular eyelet	Hick stripe eyelets		Tightly	Smooth, small perforations, thin
7	Round eyelet	Smooth, eyelet: big-small-big		Tightly	Smooth, small perforations, dense
9	Round eyelet	Smooth, eyelet: small-none-small		Sparsely	Smooth, small perforations, Thin
10	Round eyelet	Smooth, eyelet: small-big-small		Tightly	Smooth, small perforations, dense

## 2 Discussion

Pollen is one of the more conservative organs of plants.

Studies have shown that the morphological characteristics of pollen grains, especially the outer wall decoration, size, the length of germination groove, and the size of perforation, could be used as one of the indicators to calculate the evolution degree of species (Ertman, 1978), and could also be used as the basis for inter-species identification. It has been applied to the variety identification of *Eucalyptus robusta* (Wu et al., 2012), *Actinidia chinensis* (Qi et al., 2017), *Paeonia suffruticosa* (Wei, 2007), *Lonicera japonica* (Zhou et al., 2016), *Malus spectabilis* (Zhang et al., 2018), and *Cerasus avium* (Wei et al., 2014). Studies on pollen morphology of *Hydrangea* genus and its related genera showed that the pollen morphology of *Hydrangea* is complex, and the variation range of pollen morphology covers the variation range of pollen morphology of its related genera. The variation of pollen shape, equatorial surface characteristics, pollen outer wall decoration and pollen size in the species of *Hydrangea* is relatively stable, while interspecies are obvious (Zhang et al., 2019). Therefore, it is possible to identify the species or varieties of plants in *Hydrangea* according to the morphological characteristics of pollen.

The study of Lu and Xiong (2010) showed that the perforation size, germination groove length, pollen grain size and decoration characteristics were important shape indexes in the identification and analysis of *Chimonanthus praecox* varieties by pollen. Chen et al. (2007) suggested that the pollen evolution trend was from small to large, shape evolution was from prolate to ultra-prolate, the longer the pollen grains, the higher the degree of evolution. In this study, we found that the pollen grains of *Hydrangea macrophylla* were prolate with the help of SEM. Although the pollen grains of infertile flowers 'Endless summer' and 'Otaksa' were ultra-prolate, there was no significant difference, indicating that *Hydrangea macrophylla* was one of the highly evolved species, and the shape of pollen grains cannot be used as a basis for the identification of these ten *Hydrangea* varieties. This was inconsistent with previous studies that pollen morphology and size characteristics can be used as a basis for variety identification (Xue et al., 2012; Xiao et al., 2014). This may be due to the complex genetic background of the *Hydrangea macrophylla*, and the current horticultural varieties are obtained through multiple generations of hybridization.

Many studies have shown that the development process of the pollen outer wall is smooth → small perforation → perforation → retiform → coarse retiform → wrinkled, and the germination groove is developed from three grooves to multi-grooves, to scattered perforation, and whether there are granular substances in the perforation. The data analysis and research are carried out according to the size, shape and decoration of pollen grains, which provides strong evidence for plant classification (Yuan and Wang, 2002; Liu et al., 2013; Chen et al., 2019). In this study, we found that the outer walls of pollen grains of ten *Hydrangea* varieties were all hole pattern, in which the outer walls of 'Lanarth' pollen grains were smooth and sparse. Relatively, the outer walls of 'Otaksa', 'Mona Lisa', 'Headdress flower' and 'Popcorn' pollen grains were dense and wrinkled. Therefore, it was considered that 'Lanarth' is one of the original varieties with thick leaves, strong plants and strong resistance, which is suitable for cross parent.

As far as the width of germination groove is concerned, the germination groove of 'Preziosa' is wider, and it is sunken into a boat type, which is significantly different from other varieties. This may be because that the 'Preziosa' is the hybrid offspring of *Hydrangea serrata* Ser. and *Hydrangea paniculata* 'Grandiflora', and has a distant genetic relationship with other *Hydrangea paniculata* 'Grandiflora'.

In conclusion, the pollen grain outer wall decoration of different varieties of *Hydrangea macrophylla* is very different and has a certain correlation with plant morphological characteristics and stress resistance. Although some scholars insisted that pollen morphology cannot be used as a basis for cultivar identification, it must be combined with other traits of plants or biotechnology to obtain the correct results (We, 2007), palynology also has certain reference significance in systematic classification or cultivar identification, which can be used as one of the bases for cultivar identification.

### 3 Materials and Methods

#### 3.1 Experimental materials

In this study, fresh pollen grains of *Hydrangea* varieties from the Flower Base of Hunan Agricultural University were used as materials (Table 6).

Table 6 Information of ten hydrangea varieties

Codes	Variety	Flower pattern	Inflorescence shape	Fertile flower	Infertile flower
1	'Maman rose'	Single flower	Hydrangea	Big and full buds	Big and full buds
2	'Preziosa'	Coarse hydrangea	Hydrangea	Small and full buds	Small and full buds
3	'Endless summer'	Single flower	Hydrangea	Small and un full buds	Small and un full buds
4	'Otaksa'	Single flower	Hydrangea	Small and full buds	Small and full buds
5	'Mona Lisa'	Single flower	Hydrangea	Big and full buds	Big and full buds
6	'Headdress flower'	Double flower	Hydrangea	Small and un full buds	Small, mickle and un full buds
7	'Porpcorn'	Single flower	Hydrangea	Big and full buds	Big and full buds
8	'Komachi'	Double flower	Hydrangea	Small and un full buds	Small, mickle and un full buds
9	'Lanarth'	Flat petals	Lace hat type	Big and full buds	Big, mickle and un full buds
10	'First love'	Flat petals	Lace hat type	Big and full buds	Big, mickle and un full buds

#### 3.2 Experimental methods

Flower buds that are about to mature and open were collected on a sunny morning and placed in a petri dish with dry filter paper, and the variety name was marked. Then anthers were peeled, and pollen was removed. Fresh pollen was placed under JSM-6390LV scanning electron microscope for photograph observation, photographing, measurement and statistics.

Representative visual fields were selected and photographed with 1 000× (group), 5 000× (individual form, equatorial plane, germinal groove), 10 000× (local, decoration and polar view) and Excel was used to collect data. The length (L), width (W), germination groove length and width were measured by 10 pollen grains of each variety, and the maximum and minimum values were taken. SPSS was used to calculate the average value, standard deviation and DUNCAN multiple comparison analysis. Pollen grain size was represented by length and width product (L×W); the shape of pollen grains was represented by the ratio of length to width (L/W).  $L/W \geq 2$  was ultra-prolate,  $2 > L/W > 1.5$  was prolate, and  $1 < L/W \leq 1.5$  was near prolate (Zhang et al., 2019). Pollen size and morphology, polar characteristics, germination groove characteristics (germination groove length and width of 3 pollen grains per variety) and outer wall decoration were described.

#### Authors' Contributions

CHX and ZJW were the experimental designers and executors of this study. CHX and ZJW completed the data analysis, the paper first draft writing. WXM, ZHJ, and ZJF participated in the experimental design and the analysis of experimental results. CHX was the project designer and director, guiding experimental design, data analysis, manuscript writing and revision. All authors read and approved the final manuscript.

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