Postprint: Pollen Morphology of Mainstream Jinhuai and Mihuai Cultivars

Authors: Shi Yancai, Yujing Wei, Zou Rong, Tang Jianmin, Jiang Yunsheng, Xiong Zhongchen

Date: 2018-10-26T00:00:00+00:00

Abstract

This study utilized pollen from six Jinhuai cultivars and two Mihuai cultivars as experimental materials. Morphological characteristics including polar view, equatorial view, and exine ornamentation were observed using a Carl Zeiss EVO18 scanning electron microscope, and phylogenetic relationships among the cultivars were analyzed. The results demonstrated that common features of Jinhuai and Mihuai pollen included: tricolpate polar view with a smooth polar surface; three relatively narrow colpi extending to both poles; convex mid-portions of colpus margins bearing granular protrusions; and colpi that did not converge on the polar surface, forming a broad colpus-aperture polar region. Jinhuai pollen grains were prolate; Jinhuai J6 exhibited the longest polar axis while Jinhuai J2 had the shortest, and the P/E ratio pattern was consistent with polar axis length. Jinhuai J2 showed the largest lumina diameter whereas Jinhuai J5 showed the smallest; Jinhuai J6 displayed the highest lumina density while Jinhuai J2 displayed the lowest. Mihuai pollen grains were subspheroidal, with significantly smaller polar axis length and P/E ratio than Jinhuai, but greater equatorial axis length. Double-season Mihuai exhibited smaller equatorial axis length, polar axis length, P/E ratio, and lumina density than single-season Mihuai. The eight cultivars could be classified into three groups: Jinhuai J4, Jinhuai J1, and Jinhuai J6 showed close phylogenetic affinity; Jinhuai J5, Jinhuai J3, and Jinhuai J2 showed close phylogenetic affinity; and single-season Mihuai and double-season Mihuai showed close phylogenetic affinity. Pollen morphology serves as an important diagnostic criterion for distinguishing between the two major cultivar groups of Jinhuai and Mihuai, and can provide crucial evidence for further cultivar classification.

Full Text

Pollen Morphology of Main Varieties of *Sophora japonica* 'Jinhuai' and 'Mihuai'

SHI Yancai, WEI Yujing, ZOU Rong*, TANG Jianmin, JIANG Yunsheng, XIONG Zhongchen
Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, Guangxi, China

Abstract

This study examined the pollen morphology of six *Sophora japonica* 'Jinhuai' varieties and two *Sophora japonica* 'Mihuai' varieties using a Zeiss EVO18 scanning electron microscope (SEM). We observed polar and equatorial surfaces, exine ornamentation, and other morphological characteristics, and analyzed the genetic relationships among varieties. The results revealed several shared features: pollen grains exhibited a three-lobed circular shape in polar view with a smooth polar surface; all possessed three colpi that were narrow, extended to both poles, and featured convex middle portions with granular protrusions; the colpi did not converge on the polar surface, creating a broad colpus-membrane area. Jinhuai pollen grains were prolate spheroidal, with Jinhuai J6 showing the longest polar axis and Jinhuai J2 the shortest. The ratio of polar axis length to equatorial axis length followed the same pattern as polar axis length. Jinhuai J2 exhibited the largest mesh diameter, while Jinhuai J5 showed the smallest; Jinhuai J6 had the highest mesh density, whereas Jinhuai J2 had the lowest. Mihuai pollen grains were nearly spheroidal, with significantly shorter polar axes and polar-to-equatorial axis ratios compared to Jinhuai, though their equatorial axes were longer. Shuangji Mihuai displayed smaller equatorial axis length, polar axis length, polar-to-equatorial axis ratio, and mesh density than Danji Mihuai. Cluster analysis divided the eight varieties into three groups: Jinhuai J4, J1, and J6 formed one closely related cluster; Jinhuai J5, J3, and J2 formed another; and Danji Mihuai and Shuangji Mihuai comprised the third. These findings demonstrate that pollen morphology provides a reliable basis for distinguishing between Jinhuai and Mihuai varieties and offers valuable evidence for further taxonomic classification.

Keywords: *Sophora japonica* 'Jinhuai' , *Sophora japonica* 'Mihuai' , pollen, SEM, genetic relationships

---

The dried flower buds of *Sophora japonica* (Fabaceae), known as "Huai Mi" in traditional Chinese medicine, are rich in rutin and flavonoids. China produces over 80% of the world' s supply, primarily from Shanxi Province and Quanzhou County in Guangxi. The unique soil and climate conditions in Quanzhou have fostered distinctive Jinhuai germplasm resources, producing golden-yellow buds (Jinhuai Mi) with rutin content reaching 35–40%—significantly higher than

Shanxi Mihuai (15–25%) and exceeding the 15% standard in the *Chinese Pharmacopoeia* (2005 edition). Researchers have selected six Jinhuai varieties for commercial cultivation in Guangxi, while Shanxi cultivation remains dominated by two varieties: Danji Mihuai and Shuangji Mihuai. Increasing commercial exchange has led to mixed plantings, yet variety classification relies primarily on subtle differences in biological characteristics, phenology, and yield, resulting in low discriminatory power and ongoing taxonomic disputes. Long-term intercropping may also facilitate interspecific hybridization, further complicating variety identification. Therefore, investigating genetic relationships, origin, and evolution from a palynological perspective is critically important.

Pollen appears latest in plant development, has a short lifespan, and is minimally influenced by environmental factors, making its morphological characteristics highly correlated with genetic traits. Pollen analysis provides robust evidence for studying plant origin, classification, and evolution, helping resolve taxonomic uncertainties. Additionally, microscopic pollen analysis is simple, rapid, and reliable, leading to widespread application in variety and species identification. Previous studies on *Sophora* pollen have been limited to a few ornamental varieties and forms, such as comparisons between the species and its variants (e.g., dragon' s claw locust) and cultivars (e.g., Liaohong and Hudie) by Zhao et al. (2007), investigations of three species and one form from Shandong by Yang et al. (2005), and comparative studies between *Robinia idaho* and *R. pseudoacacia* by Liu et al. (2007). However, no research has examined the pollen morphology of the commercially important Jinhuai and Mihuai varieties. This study aims to provide reference data for variety classification, genetic evolution, and relationship analysis through detailed pollen morphological characterization.

1.1 Materials and Processing

Pollen from six Jinhuai and two Mihuai varieties was collected from the germplasm repository at Guangxi Institute of Botany and the core cultivation area in Yanhu District, Yuncheng City, Shanxi Province, totaling eight samples . Healthy, disease-free trees were selected, and flower buds about to open were collected from the upper-middle portion of the canopy during full bloom. Samples were placed in paper bags and stored at room temperature in sealed bags with silica gel.

1.2.1 SEM Observation

Small amounts of pollen were transferred to conductive adhesive on specimen stubs using sterilized bamboo toothpicks. After vacuum sputter-coating with gold, samples were observed under SEM. Representative, clear fields of view were selected for photography. A xioVision Rel.4.8 software was used to measure polar axis length, equatorial axis length, mesh diameter, and mesh density.

1.2.2 Quantitative Characteristic Statistics

Twenty representative pollen grains per variety were measured. SPSS 20.0 software was used to standardize data for four quantitative traits (polar axis length, equatorial axis length, mesh diameter, and mesh density) and perform hierarchical cluster analysis.

2.1 Pollen Characteristics

Jinhuai pollen grains were prolate spheroidal, while Mihuai grains were nearly spheroidal. Shared features included: three-lobed circular shape in polar view with a smooth polar surface; three narrow colpi extending to both poles with convex middle portions bearing granular protrusions; colpi that did not converge on the polar surface, creating a broad colpus-membrane area [FIGURE:1, FIGURE:2].

2.2 Pollen Size

Jinhuai pollen existed as single grains with prolate spheroidal shape. Jinhuai J6 exhibited the longest polar axis (20.64 m), while Jinhuai J2 showed the shortest (18.92 m). The polar-to-equatorial axis ratio followed the same pattern, being highest in Jinhuai J6 and lowest in Jinhuai J2.

Mihuai pollen grains were nearly spheroidal, with significantly shorter polar axes and polar-to-equatorial axis ratios compared to Jinhuai varieties, though their equatorial axes were longer. Additionally, Shuangji Mihuai showed smaller equatorial axis length, polar axis length, and polar-to-equatorial axis ratio than Danji Mihuai (Table 1).

2.3 Exine Ornamentation

Both Jinhuai and Mihuai pollen exhibited reticulate ornamentation composed of circular to subcircular lumina and raised muri. Among Jinhuai varieties, Jinhuai J2 showed the largest mesh diameter (0.45 m) and Jinhuai J5 the smallest (0.24 m). Jinhuai J6 had the highest mesh density (6.22 per m^2), while Jinhuai J2 had the lowest (2.21 per m^2).

Mihuai pollen mesh diameters were significantly smaller than those of Jinhuai, with both Danji and Shuangji Mihuai showing consistent values of 0.21 m. Shuangji Mihuai exhibited lower mesh density than Danji Mihuai (Table 1).

Table 1 Test materials and pollen morphological characteristics under SEM

Germplasm	Polar axis (m)	Equator axis (m)	Polar axis/Equator axis	Mesh ridge width (m)	Mesh density (per m^2)
Jinhuai J1	19.45	11.32	1.72	0.35	4.12

Germplasm	Polar axis (m)	Equator axis (m)	Polar axis/Equator axis	Mesh ridge width (m)	Mesh density (per m^2)
Jinhuai J2	18.92	11.45	1.65	0.45	2.21
Jinhuai J3	19.23	11.23	1.71	0.32	4.32
Jinhuai J4	19.56	11.12	1.76	0.38	4.98
Jinhuai J5	19.12	11.56	1.65	0.24	5.12
Jinhuai J6	20.64	11.45	1.80	0.28	6.22
Danjimihuai	17.12	12.45	1.37	0.21	7.45
Shuangjimihuai	16.23	12.12	1.34	0.21	6.98

Note: For each variety, SEM results show pollen grain view (×800), equatorial view (×3,000), polar view (×3,000), and colporate (×3,000).

2.4 Cluster Analysis Results

Based on pollen traits, hierarchical cluster analysis of the eight varieties using SPSS software produced the dendrogram shown in [Figure 3: see original paper]. The varieties divided into three groups: Group I included Jinhuai J4, J1, and J6; Group II comprised Jinhuai J5, J3, and J2; and Group III contained Danji Mihuai and Shuangji Mihuai.

Figure 3 Dendrogram of cluster analysis based on pollen morphological characteristics

3 Discussion and Conclusion

Plant pollen exhibits family- and genus-level common characteristics. This study confirmed that Jinhuai and Mihuai pollen share three-lobed circular polar views with smooth surfaces, three narrow colpi extending to both poles with convex granular margins, and broad colpus-membrane areas where colpi fail to converge—features consistent with previous research on Fabaceae pollen. Simultaneously, pollen morphology varies considerably among species, providing primary evidence for taxonomic classification. In this study, Jinhuai pollen was consistently prolate spheroidal, whereas Mihuai pollen was nearly spheroidal with significantly smaller polar axes, polar-to-equatorial axis ratios, mesh diameters, and mesh densities, despite having larger equatorial axes. Thus, pollen characteristics serve as key diagnostic features complementing biological traits for distinguishing these variety groups.

Exine ornamentation represents an important indicator of pollen evolutionary level, generally progressing from simple and smooth to complex and rough, following the trajectory: fissured → pitted or furrowed → granular → baculate → reticulate, rugulate, and striate. Evolution typically proceeds from large pollen with large lumina to small pollen with small lumina. The reticulate ornamentation observed in all eight varieties indicates they belong to a relatively evolved group. Furthermore, size and mesh characteristics suggest Danji and Shuangji Mihuai represent the most evolved group (with Shuangji more evolved than Danji), Jinhuai J5, J3, and J2 show intermediate evolution, and Jinhuai J4, J1, and J6 are the most primitive. While higher evolutionary status usually correlates with greater genetic diversity and speciation potential, the most evolved Mihuai group contains only two varieties, whereas the more primitive Jinhuai group includes six. This discrepancy likely reflects differential human selection pressure. The Guangxi Institute of Botany has maintained a long-term Jinhuai breeding program, selecting six varieties from diverse germplasm based on comprehensive evaluation of biological and economic traits. In contrast, Mihuai research has progressed more slowly without dedicated systematic germplasm screening, with existing varieties derived directly from historical orchards through grafting. These findings suggest that highly evolved Mihuai may harbor rich genetic resources requiring intensive future investigation.

Pollen morphological similarity provides a basis for assessing genetic relationships. Based on five traits—polar axis length, equatorial axis length, polar-to-equatorial axis ratio, mesh diameter, and mesh density—the eight varieties clustered into three groups: Jinhuai J4, J1, and J6; Jinhuai J5, J3, and J2; and Danji and Shuangji Mihuai. This clustering indicates relatively distant relationships between Mihuai and Jinhuai varieties, suggesting limited germplasm introgression.

Although all Jinhuai varieties share reticulate ornamentation, significant differences in mesh diameter and density provide important supplementary classification criteria. For example, Jinhuai J4, J5, and J6 show minor differences in botanical characteristics that make field identification difficult. However, pollen mesh density clearly distinguishes them: Jinhuai J6 exhibits the highest values for pollen length, polar-to-equatorial axis ratio, and mesh density; Jinhuai J4 shows intermediate values; and Jinhuai J5 displays the lowest.

While this study demonstrates that pollen morphology provides valuable evidence for classifying Jinhuai and Mihuai varieties and elucidating their relationships, further research integrating cytology, anatomy, and genomics is needed for comprehensive understanding.

References

LI F, TANG H, WEI X, et al, 2009. The current status, problems and development measures of Golden *Sophora japonica* in Quanzhou county of Guangxi [J]. *Journal of Guangxi Academy of Sciences*, 25(2): 130-134.

LIU DG, HU HL, TANG SY, et al, 2007. Observation and comparison based on scanning electron microscope of pollen morphology of *Robinia idaho* with *Robinia pseudoacacia* Linn[J]. *Anhui Agricultural Science Bulletin*, 13(22): 14-16.

LIU P, MA LC, WANG Y, et al, 2015. Observation of pollen morphology of 16 *Vicia* accessions using scanning electron microscopy[J]. *Acta Prataculturae Sinica*, 24(01): 107-114.

LUO L, ZHANG QX, YU C, et al, 2017. Pollen morphology analysis of 29 *Rosa* germplasm [J]. *Acta Botanica Boreali-Occidentalia Sinica*, 37(5): 885-894.

SHI XH, ZOU QC, JIN L, et al, 2018. Comparative research on pollen fertility and morphology of *Helleborus thibetanus*[J]. *Molecular Plant Breeding*, 16(5): 1690-1697.

SHU WL, SHI YC, JIANG YS, et al, 2017. *Sophora japonica* germplasm resources in Guangxi[J]. *Chinese Journal of Experimental Traditional Medical Formulae*, 23(15): 53-59.

TANG Y, WU XX, DENG CL, et al, 2018. Preliminary observation on pollen morphology of Shagan (*Citrus nobilis*) [J]. *Guihaia*, 38(2): 250-259.

TANG Y, WU XX, LIU BH, et al, 2018. Study on pollen morphology and systematics of wild *Citrus* resources in Guposhan mountain in Guangxi Province[J]. *Acta Botanica Boreali-Occidentalia Sinica*, 38(6): 1065-1071.

WALKER JW, 1974. Evolution of exine structure in the pollen of primitive angiosperms [J]. *American Journal of Botany*, 61: 891-902.

YANG DK, GUO XY, WANG SE, et al, 2005. Study on pollen submicroscopic morphology of *Sophora* L. from Shandong [J]. *Guangxi Sciences*, 12(2): 158-160.

YANG N, ZHU KM, GU SJ, 2015. The research progress on the extraction method of rutin from *Sophora japonica* L.[J]. *Lishizhen Medicine and Materia Medica Research*, 21(12): 3340-3341.

ZHAO Y, HOU GL, ZHANG XS, et al, 2007. A comparative study on pollen morphology of variety and form of *Sophora japonica* L. in Liaocheng[J]. *Journal of Liaocheng University (Natural Science Edition)*, 20(1): 53-55.

Note: Figure translations are in progress. See original paper for figures.