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Combined Morphological and Palynological Classification for *Hibiscus syriacus* L. (Malvaceae): Construction of the Diagnostic Classification Framework and Implications of Pollen Morphological Variation on Fruiting

Fen Xiao [1,2], Xiaohong Wang [1,2,*], Yun Jiang [3], Chulin Chen [1], Jiajia Chen [1], Jingwen Zhang [1] and Yafeng Wen [1,2]

1 College of Landscape Architecture, Central South University of Forestry & Technology, Changsha 410004, China
2 Hunan Big Data Engineering Technology Research Center of Natural Protected Areas Landscape Resources, Changsha 410004, China
3 Department of Horticulture and Landscape, Shanghai Chenshan Botanical Garden, Shanghai 201602, China
* Correspondence: t19960222@csuft.edu.cn; Tel.: +86-156-1633-1155

Abstract: Identifying useful taxonomic indicators for classifying *Hibiscus syriacus* L. (Malvaceae) cultivars can help address challenges in their homonymy and synonymy. Moreover, analyzing which pollen traits possibly lead to their successful fruiting can serve to guide the hybridization and breeding of *H. syriacus*. For the first time, this study classified 24 cultivars of *H. syriacus* based on 24 morphological and palynological indicators assessed for flowers, leaves, and pollen grains. These indicators were a mixture of quantitative and qualitative traits, measured to contribute to the identification and classification of *H. syriacus* cultivars. The results showed that the 24 *H. syriacus* cultivars could be classified into 2–6 clusters according to different taxonomic criteria. The leading diagnostic indicators were eight quantitative and eight qualitative traits, of which two new quantitative traits—the width of the spine base (SW) and average of the pollen grain radius and spine length (D-spine)—and five new qualitative traits—the amount of pollen surface spines (O-SA), whether the petals have the red center (B-RC), whether the pollen surface ruffles strongly (B-RS), the degree of pollen surface ruffling (O-DR), and relationship between calyx and bract (O-CB)—could be used as defining traits for *H. syriacus* cultivars owing to their robust contribution to the classification. The correlations between indicators for flowers, leaves, and pollen grains were explored, which revealed that the O-SA in *H. syriacus* was strongly tied to quantitative pollen traits. Furthermore, three qualitative morphological traits—whether the stamens are heterogeneous in terms of inner petals (B-IP), O-CB, and whether the leaf lobing is strong (B-LL)—were correlated with partial quantitative pollen traits. We also found that those *H. syriacus* cultivars with micro-spines or granulate on the pollen grain surface have higher fruiting rates; additionally, pollen diameter, spine length, and spine spacing might also be potential factors influencing successful breeding. The insights gained from this study could fill a key knowledge gap concerning the taxonomic criteria suitable for distinguishing *H. syriacus* cultivars. Our findings also provide timely information on how to understand the pollination process, especially those aspects leading to pollinator selection via pollen grain features, which could influence breeding programs and outcomes.

Keywords: classification; *Hibiscus* L.; morphology and palynology; pollen morphology variation; quantitative and qualitative traits

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1. Introduction

Hibiscus syriacus L., a species of the genus *Hibiscus* of the Malvaceae family, is one of the traditional Chinese flowers and the national flower of South Korea [1,2]. It is noted for its diverse floral morphological characteristics, wide distribution, and adaptability [3].

Approximately 250 cultivars of *H. syriacus* are known worldwide [2]; however, suitable cultivars for horticultural landscapes are few and limited [4], and still beset with challenges of homonymy and synonymy. Therefore, a unified nomenclature and classification of *H. syriacus* cultivars is of particular importance to maximize the use of *H. syriacus* resources.

Only few studies have attempted to classify *H. syriacus* cultivars in terms of differing color systems or pollen grains [2,5]. The spiny pollen grains of the Malvaceae are beneficial for the classification of this group, mainly for *Hibiscus* species, as demonstrated for *H. pernambucensis* and *H. tiliaceus* by the study of Oliveira et al. [6]. Accordingly, for *H. syriacus* cultivars that are homochromatic and have similar phenotypic characteristics, palynological classification must be very useful. Sung et al. classified 22 *H. syriacus* cultivars into six clusters based on six pollen feature indices [5]. We hypothesized that relying solely on pollen traits for plant taxonomy is insufficient and not intuitive enough for a species whose pollen morphology is relatively uniform. Instead, it seems more sensible and acceptable to arrive at a quick classification directly gleaned from an examination of external morphology, such as flower and leaf traits; however, that might not always be compatible with palynological investigations. Taxonomic studies of this species in other aspects such as palynological or molecular classification remain surprisingly limited. In particular, a classification that integrates all aspects of morphology, such as the combination of flower, leaf, and pollen features, is not yet available. Such studies are necessary as they will allow a more intuitive and rapid classification of most *H. syriacus* cultivars and will be essential for marketability and industrial development.

Pollen morphology has a unique structure determined by the genes of each species, meaning it is reliable, quite stable, and generally unaffected by the external environment [7,8]. Accordingly, it is often used in plant taxonomic and palynological analyses for identifying and classifying genera, species, or cultivars of horticultural plants [9–11]. In general, the pollen grains with *H. syriacus* are apolar, spheroidal monads. Quantitative pollen traits were employed to analyze the morphology of *H. syriacus* pollen grains in studies by Sung et al. (pollen diameter, spine exine length, no. of spine exines, and distance between spine exines) and Zhao et al. (spine length, width of spine base, spine distance, pollen diameter, spine length/width of spine base, and pollen diameter/spine length), and both found significant differences in spine features among cultivars [3,5]. However, taking this approach alone is inadequate; other relevant numerical traits must also be investigated, such as the exact pollen shape and the pollen diameter in two directions in the two-dimensional plane (X-axis and Y-axis), which are collectively needed to inform and analyze the variation in pollen morphology among cultivars of *H. syriacus*. Quantitative morphological traits of pollen grains are widely used in plant numerical classification [11,12]. Pollen grains are also diverse in exine sculpturing. However, related qualitative traits, such as pollen ornamentation and perforation, are mostly found in textual descriptions of general pollen morphology and are rarely involved in the numerical taxonomy of pollen grains using coding methods.

The same is possible for phenotypic characteristics of *H. syriacus*. We have already performed a preliminary classification for *H. syriacus* based its quantitative and qualitative floral morphological traits [13]; however, the contribution of its qualitative indicators to the classification framework has not been evaluated yet. Accordingly, in this study, we aimed to detect classification criteria by considering diverse morphological traits of a combination of plant parts, namely flowers, leaves, and pollen grains, which could enhance the credibility of the classification and improve its efficiency. Moreover, in general, research on reliable qualitative indicators suitable for plant numerical classification is still limited. If they are quantified and coded, a better classification framework can be produced than traditional ones, which can facilitate the establishment of new taxonomic criteria, in turn [14]. In earlier work, Fen et al. demonstrated that qualitative traits contributed substantially to a robust classification within conifer pine *Cathaya argyrophylla* [14]. Hence, we sought to apply the same methodology to *H. syriacus* in the present study.

Pollen export might help to form specific floral characteristics that attract pollinators [15], thereby indirectly influencing plant fruiting and seed set rates. However, limited knowledge is available on the co-varying relationships between the pollen morphology, flower morphology, and the fruiting rate of *H. syriacus*. Insect pests might be among the drivers of diversity in seed set levels among *H. syriacus* cultivars. Additionally, the effects of pollen traits on the pollination mechanism or pollinator selection, or both, might be another reason for it, yet these have not been extensively studied. The available evidence suggests that the relationship between pollen and fertility is complex because the whole developmental mechanism is jointly influenced by many factors, such as pollen's grain size, spine length, and spacing, and even the pollinator species or their ability to carry certain amounts of pollen [16,17].

In this study, 24 morphological and palynological indicators were used together to classify 24 cultivars of *H. syriacus*, revealing parsimonious criteria for the first time. The variation in pollen morphology among cultivars was also assessed, as well as the contribution of qualitative traits to the classification. We detected possible links between pollen traits affecting the fruiting rate for *H. syriacus*. Altogether, these findings could fill a knowledge gap concerning the classification of *H. syriacus* cultivars and provide further insight into pollen features leading to the successful fertility enhancement of *H. syriacus*.

2. Materials and Methods

2.1. Plant Materials and Sources

A total of 24 *H. syriacus* cultivars were collected from Hunan, Henan, Zhejiang, and Shanghai, China, as well as from South Korea and the USA, and a resource garden was established at the Central South University of Forestry and Technology (Table 1). From selected healthy, clean, pest-free buds, and incompletely bloomed or blooming flowers, the petals were separated from the anthers, with the pollen grains obtained from the anthers using a laboratory blade. All these operations were carried out in a sterilized chamber and on sulfate paper. Five individual plants per cultivar were taken and mixed together as one composite sample. The collected pollen grains were dried under room conditions for 1 day, placed in centrifuge tubes, and immediately subjected to a scanning electron microscope, and remaining pollen materials were stored in a refrigerator at 4 °C.

Table 1. Information about the 24 *Hibiscus syriacus* cultivars.

No.	Cultivar	Source	No.	Cultivar	Source
1	Marina	US	13	Purple pillar	US
2	White chiffon	SH	14	Rubis	KR
3	Pink giant	SH	15	Chungmu	KR
4	China chiffon	SH	16	Suminokurahanagasa	KR
5	Paeoniflorus	HN	17	Pyonghwa	KR
6	Woodbridge	SH	18	Blue bird	US
7	Diana	SH	19	Akagionmamori	KR
8	Lavender	SH	20	Red heart	SH
9	Hamabo	SH	21	Qiancenghong	HEN
10	Elegantissimus	SH	22	Hongyun	HEN
11	Arang	HN	23	Huaban	HEN
12	Lavandula chiffon	SH	24	Naesarang	KR

Note: SH: Shanghai, China; HN: Hunan Province, China; HEN: Henan Province, China; KR: South Korea; US: the United States.

2.2. Scanning Electron Microscopy (SEM) and Coding the Pollen Qualitative Traits

The collected pollen grains were evenly spread on a sampling tray, then sprayed with metal (SCD 500) and positioned under a scanning electron microscope (JSM 6360-LV) operating at a voltage of 10.0 kV for observation, and electromicrographed at magnifications of 150×, 700×, 1000×, and 1600×. For each cultivar, 10 mature, well-formed pollen grains were randomly selected for measurement. In total, 240 pollen grains were measured.

A total of 12 pollen trait indicators were chosen for quantification and encoded using the scalar quantifying method (Table 2) [18], including eight quantitative traits ("N"), which are numerical and calculated in raw data form without coding. Of these, width of the spine base (SW), spine index (SL/SW), and the average pollen grain radius and spine length (D-spine) were evaluated for the first time for *H. syriacus*. Two binary traits ("B"), coded by "0" or "1", denoted negative and positive states, respectively. Two ordered multistate traits ("O") were each coded with consecutively arranged positive integers ("1", "2", "3", . . .). All four qualitative traits were new indicators (Table 2) never assessed before in *H. syriacus* cultivars, as were the quantified characteristics used to describe their degree of pollen surface ruffling and the number of spines. The pollen description terminology used in this study follows that developed by Erdtman et al. [19], Wang et al. [20], and Punt et al. [21].

Table 2. Twelve pollen traits and their codes for *Hibiscus syriacus*.

No.	Characteristic	Code Type	Code Details
1	Pollen diameter parallel to the X-axis (D_1)	N	/
2	Pollen diameter parallel to the Y-axis (D_2)	N	/
3	Pollen shape ratio (D_2/D_1)	N	/
4	Length of the spine (SL)	N	/
5	Width of the spine base (SW)	N	/
6	Spine index (SL/SW)	N	/
7	Radius of the pollen grain and spine length, averaged (D-spine)	N	/
8	Spacing between spines (S-spine)	N	/
9	Whether the pollen surface has micro-spines or granular verrucae (B-GW)	B	Yes, 1; No, 0
10	Whether the pollen surface ruffles strongly (B-RS)	B	Yes, 1; No, 0
11	Number of pollen surface spines (O-SA)	O	Few (<40), 1; Medium (40–50), 2; Many (≥50), 3
12	Degree of pollen surface ruffling (O-DR)	O	Smooth and largely unruffled, 1; Light ruffles, 2; Strong ruffles, 3

Note: D_1 refers to the diameter parallel to the X-axis in the observation view; D_2 refers to the diameter parallel to the Y-axis in the observation view; the three classes of O-SA were determined by the average range values of pollen spine numbers of 24 *H. syriacus* cultivars (30–60).

2.3. Flower and Leaf Morphological Indicators and Their Measurement

Eleven flower morphological indicators were employed in this study, consisting of seven numerical ("N") characteristics, two binary ("B"), and two ordered multistate ("O") characteristics (Table 3). These 11 floral morphological traits were used in our previous morphological classification study of 27 *H. syriacus* cultivars [13]. However, how they are each defined, and consequently their contribution to the classification, are reported and evaluated here for the first time. An indicator of the degree of leaf lobing was used as a binary trait and applied here to *H. syriacus* (Table 3). The investigation methodology had three components [2]: (1) MS: Survey of individual measurements of the subject plant and its parts. (2) VG: Observation survey with one overall observation of the subject plant and its parts. (3) VS: Observation survey with individual observations of the subject plant and its parts.

2.4. Data Analysis

The mean, maximum, minimum, and coefficient of variation (CV) of eight numerical pollen traits were analyzed for each cultivar (Table A1). Here, the pollen shape ratio (D2/D1), which corresponds to the P/E (the length of the polar axis/equatorial diameter) indicator for polar pollen grains, was classified according to the criteria proposed by Erdtman [22]: oblate spheroidal (0.89–0.99), spheroidal (1.00), prolate spheroidal (1.01–1.14), and subprolate (1.15–1.33). To compare the 24 cultivars in terms of the eight quantitative pollen traits, a multivariate analysis of variance (MANOVA) based on the Shapiro–Wilk normality test was used, followed by one-way analysis of variance (ANOVA), and with Tukey's HSD test to determine which of the 24 cultivars differed from each other.

Table 3. Twelve flower and leaf traits and their codes for *Hibiscus syriacus*.

No.	Characteristic	Investigation Method	Code Type	Code Details
1	Stalk length (ST)	MS	N	/
2	Petal length (PL)	MS	N	/
3	Petal width (PW)	MS	N	/
4	Petals index (PL/PW)	MS	N	/
5	Red center length (RC)	MS	N	/
6	Length of red center line (RCL)	MS	N	/
7	Red center index (RCL/RC)	MS	N	/
8	Whether the stamens are heterogeneous in terms of inner petals (B-IP)	VS	B	Yes, 1; No, 0
9	Whether the petals have a red center (B-RC)	VS	B	Yes, 1; No, 0
10	Relationship of the calyx with the bract (O-CB)	VS	O	Shorter, 1; Near Equal Length, 2; Beyond, 3
11	Relationship of the red center line with the red center (O-RC)	VS	O	Near Equal Length, 1; Beyond, 2; Beyond Obvious, 3
12	Whether the leaf lobing is strong (B-LL)	VG	B	Yes, 1; No, 0

Next, bivariate correlations among the 12 pollen traits were examined using Pearson's r coefficient. To explore the developmental patterns between phenotypic features of *H. syriacus*, the associations between pollen, flower, and leaf morphological indicators were investigated. We also collected data on the fruiting rate of 11 *H. syriacus* cultivars (Table A2) [2], to test for pollen morphology effects on fruiting rates based on the correlation analysis.

A classification framework based on the 24 combined morphological indicators (Tables 2 and 3) was applied to the 24 *H. syriacus* cultivars. To do this, we first standardized (STD) the raw data for each of the 24 traits to eliminate differences in their dimensions. To this STD dataset, a principal component analysis (PCA) of 24 traits was applied, which yielded new uncorrelated variables (PCs) [23–25]. Next, variables were compared in the R-type clustering analysis (between-groups linkage as the clustering method and Pearson's r as a correlation measure), and each variable was placed as a unit in a cluster. In the Q-type analysis (Ward's method as the clustering method and squared Euclidean length as the distance measure), samples were compared using uncorrelated or least correlated variables (implemented in R-type clustering) [26]. Based on the clustering results, it was possible to identify whether the qualitative indicators helped to distinguish *H. syriacus* cultivars. All calculations and analyses described above were carried out using SPSS Statistics 19.0 software.

3. Results

3.1. Floral and Leaf Morphology of 24 H. syriacus Cultivars

The *H. syriacus* cultivars were evidently morphologically diverse. Two of the 24 cultivars in this study lacked a red center (White chiffon and Diana). All seven numerical traits varied considerably among the cultivars: ST (range: 4.00~36.30 mm; mean: 12.46 mm), PL (range: 39.60~70.60 mm; mean: 49.77 mm), PW (range: 22.20~52.30 mm; mean: 36.85 mm), and PL/PW (range: 1.15~1.81; mean: 1.39). They also varied considerably among those cultivars with a red center and red center line: RC (range: 7.30~19.60 mm; mean: 10.25 mm), RCL (range: 7.40~37.90 mm; mean: 14.51 mm), and RCL/RC (range: 1.00~2.30 mm; mean: 1.28 mm). In addition, significant differences were found among cultivars with respect to the presence or absence of inner petals (B-IP), the relationship of the calyx with the bract (O-CB), the relationship of the red center line with the red center (O-RC), and the degree of leaf lobing (B-LL) (Figure 1).

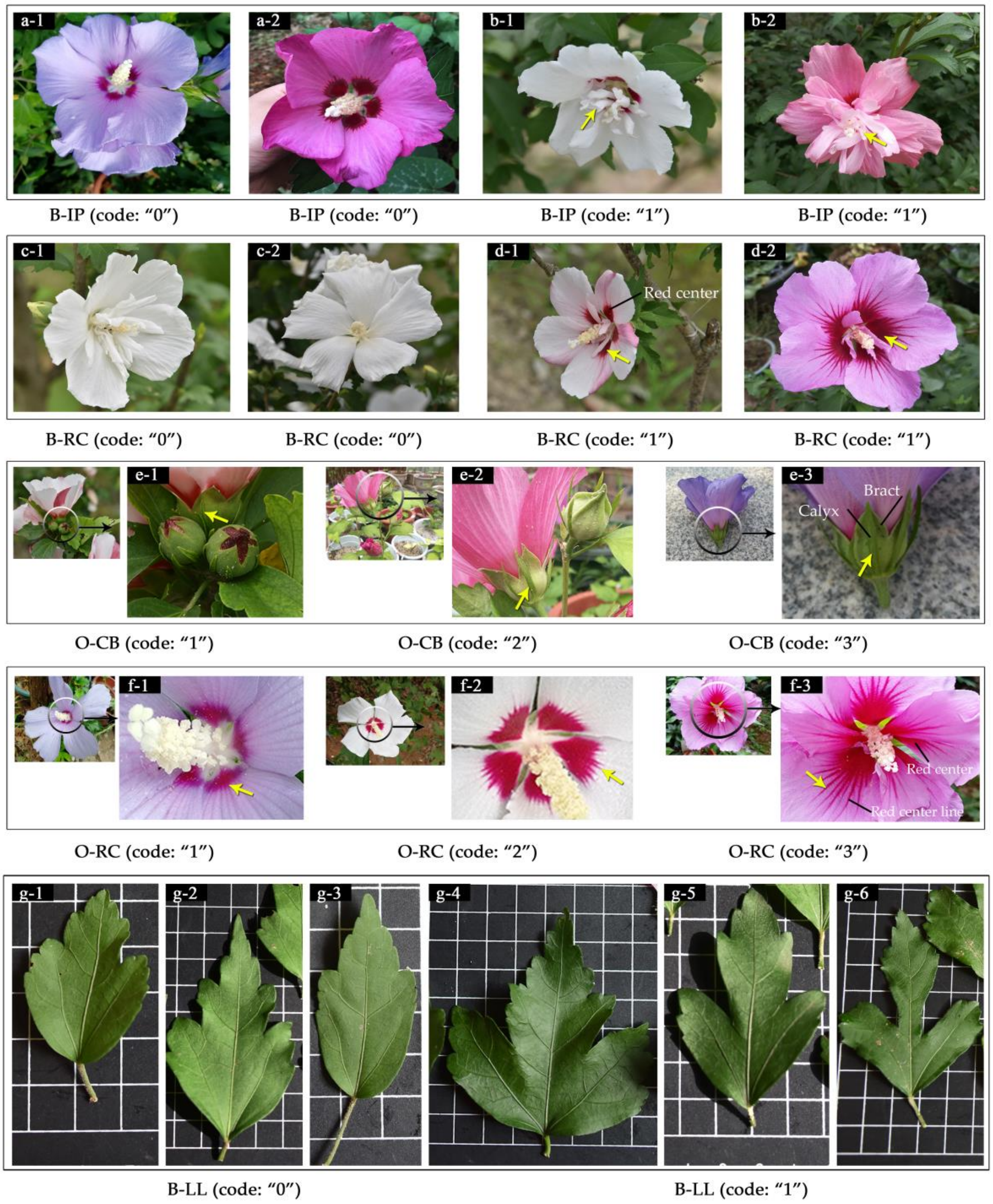

Figure 1. The description and codes for five qualitative traits of *Hibiscus syriacus* flower and leaf parts, for which the yellow arrow indicates the typical characteristic. Whether the stamens are heterogeneous in terms of inner petals (B-IP): (**a-1**,**a-2**) stamens not differentiated into inner petals (No, 0); (**b-1**,**b-2**) stamens differentiated into inner petals (Yes, 0). Whether the petals have the red center (B-RC): (**c-1**,**c-2**) petals without the red center (No, 0); (**d-1**,**d-2**) petals with the red center (Yes, 0). Relationship of the calyx with the bract (O-CB): (**e-1**) calyx shorter than bract (Shorter, 1); (**e-2**) calyx almost equal to bract (Near equal length, 2); (**e-3**) calyx longer than bract (Beyond, 3). Relationship of the red center line with the red center (O-RC): (**f-1**) red center line is almost equal to red center (Near equal length, 1); (**f-2**) red center line is longer than red center (Beyond, 2); (**f-3**) red center line is significantly longer than red center (Beyond obviously, 3). Whether leaf lobing is strong (B-LL): (**g-1**,**g-2**,**g-3**) low degree of leaf lobing (No, 0); (**g-4**,**g-5**,**g-6**) high degree of leaf lobing (Yes, 1).

3.2. Overall Pollen Morphology of *H. syriacus*

The electromicrographs obtained via SEM were used to observe and illustrate the pollen morphology of *H. syriacus* samples (Figure 2). Most *H. syriacus* pollen grains were subspherical monads with a symmetrical structure, having a spiny pollen surface with blunt spine ends. The germination pores were panpori (following the pollen terminology of Erdtman et al. and Wang et al. [19,20]). The pollen surface appeared smooth or ruffled, with varying degrees of ruffling, and the ornamentation was mostly striate, cerebroid, or a mixture of striate and cerebroid. All studied samples differed markedly in terms of pollen spines, while their pollen shapes were less variable.

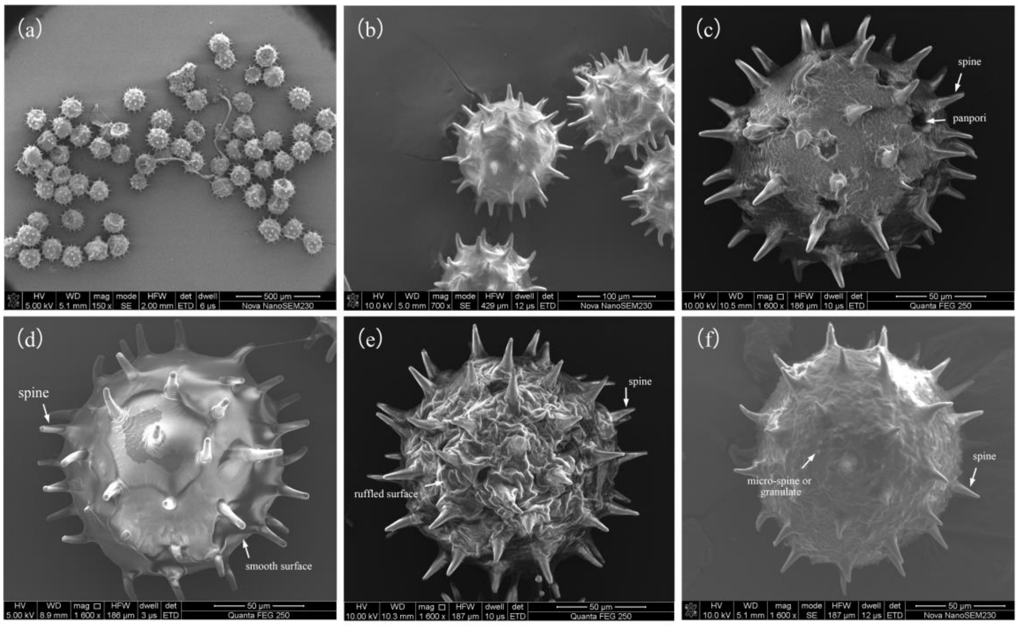

Figure 2. *Hibiscus syriacus* pollen grains electromicrographed by SEM. (**a**,**b**) An overall full view of pollen grains as spheroidal monads with spines. (**c**) The germinal porus (panpori) on the pollen surface. (**d**) A pollen grain with a smooth surface (pollen surface ornamentation is inconspicuous). (**e**) A pollen grain with a ruffled surface (pollen surface with distinctive cerebroid ornamentation). (**f**) A pollen grain with micro-spines or granulate on its surface.

3.2.1. Quantitative Traits of *H. syriacus* Pollen Grains

Details of the quantitative pollen traits of *H. syriacus* are presented in Table A1 and Figure 3. For polar pollen grains, the equatorial diameter (E) is commonly used to evaluate species' pollen size [27,28]. Here, pollen diameter parallel to the X-axis (D_1) is equivalent to this indicator. For the 24 cultivars of *H. syriacus*, the D_1 and pollen diameter parallel to the Y-axis (D_2) had mean values of 124.19 μm and 124.85 μm, respectively, ranging from 111.33 to 144.34 μm and 112.39 to 146.03 μm. The D_1 (90.19–160.95 μm) and D_2 (90.90–158.22 μm) values for the 240 measured pollen grains varied more significantly (Figure 3a,b). The exact pollen shape (D_2/D_1) of *H. syriacus* was assessed here for the first time, revealing less variation in this characteristic. For the 24 cultivars of *H. syriacus*, the pollen shape (D_2/D_1) was frequently oblate spheroidal (38%) and prolate spheroidal (46%) with a mean value of 1.01, ranging from 0.95 to 1.07. For the 240 measured pollen grains, the pollen shape likewise mostly presented as oblate spheroidal (36%) and prolate spheroidal (54%). The D_2/D_1 reached its maximum in the cultivar White chiffon (1.18), and its minimum in the cultivar Woodbridge (0.73) (Figure 3c).

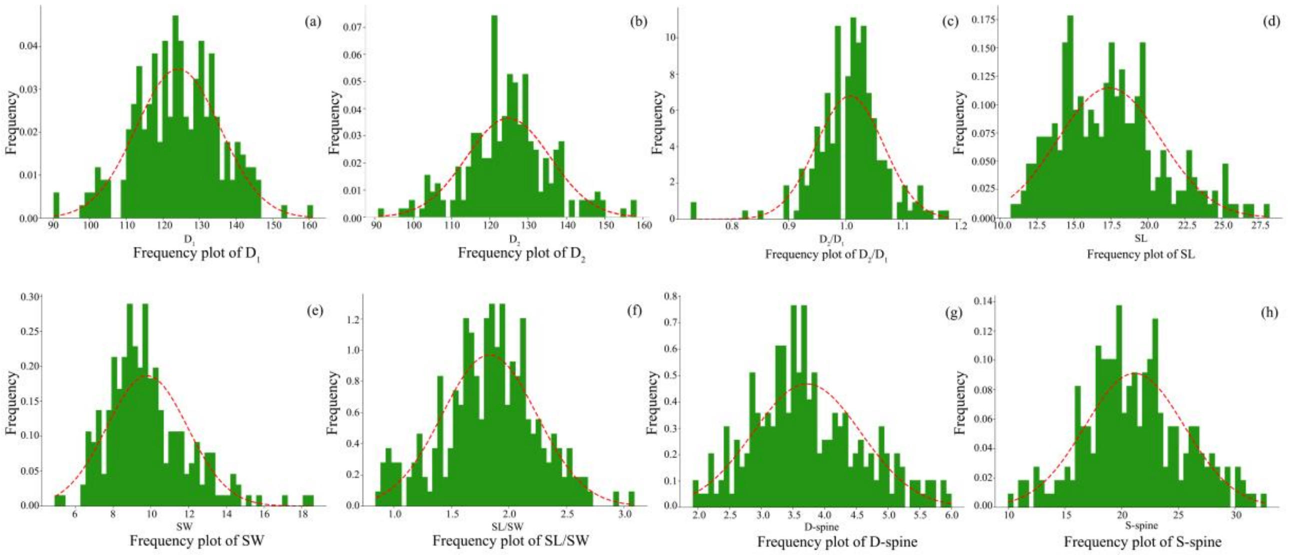

Figure 3. Histograms of eight quantitative traits of *Hibiscus syriacus* based on 240 measured pollen grains. Shown is the normalized frequency of D_1, D_2, D_2/D_1, SL, SW, SL/SW, D-spine, and S-spine for each sample of each *H. syriacus* cultivar. (**a**) The D_1 ranged from 90.19 to 160.95 μm, peaking at 120~130 μm. (**b**) The D_2 ranged from 90.90 to 158.22 μm, peaking at 120~130 μm. (**c**) The D_2/D_1 ranged from 0.73 to 1.18, peaking at 1.0~1.05. (**d**) The SL ranged from 10.73 to 28.24 μm, peaking at 17.00~17.50 μm. (**e**) The range value of SW ranged from 6.91 to 18.61 μm, peaking at 9.00~10.00 μm. (**f**) The SL/SW ranged from 0.84 to 3.09, peaking at 1.50~2.00. (**g**) The D-spine ranged from 1.92 to 6.00 μm, peaking at 3.50~4.00 μm. (**h**) The S-spine ranged from 9.98 to 32.75 μm, peaking at 20~25 μm.

Length of the spine (SL) was 17.40 μm, on average, and varied widely among the 24 *H. syriacus* cultivars (13.42~25.04 μm), especially across the 240 measured samples (10.73–28.24 μm) (Figure 3d). The width of the spine base (SW) is described here for the first time. This trait measured 9.80 μm on average, ranging from 8.25 to 15.00 μm among the 24 cultivars, while showing a near four-fold difference across the 240 samples (4.91~18.61 μm) (Figure 3e). The spine index (SL/SW) was 1.83, on average, ranging from 1.06 to 2.29 among the 24 cultivars, while spanning from 0.84 to 3.09 among the 240 studied pollen grains (Figure 3f).

The average of pollen grain radius and spine length (D-spine) was first used to describe the pollen characteristic for *H. syriacus*. D-spine values ranged from 2.22 to 5.38, with a mean of 3.67; for the 240 pollen grains of *H. syriacus*, the D-spine attained its maximum in the cultivar Woodbridge (6.00) and its minimum in the cultivar Naesarang (1.92) (Figure 3g). The spacing between spines (S-spine) was also determined in this study, having a mean value of 21.14 μm (12.85~27.37 μm) among the 24 cultivars. Across the 240 pollen grains, S-spine was largest in the cultivar Elegantissimus (32.75 μm) and smallest in the cultivar Pink giant (9.98 μm) (Figure 3h).

3.2.2. Qualitative Traits of *H. syriacus* Pollen

Four new qualitative traits were employed to describe the characteristics of the pollen surface for *H. syriacus* (Figure 4). We found relatively few cultivars with micro-spines or granulate verrucae, these amounting to 20.83% of the samples (Figure 4(a-1,a-2,b-1,b-2)). The spine numbers varied considerably between cultivars (30~54) (Figure 4c,(d-1,d-2),e), being greatest in the cultivar Pink giant. We divided this trait into three classes; most of the cultivars (70.83%) featured a lower number of spines. In addition, a significant difference in pollen surface ruffling was found among the cultivars, with ruffled surfaces predominating. Two qualitative indicators were used to code their ruffling features (Figure 4f,g,(h-1,h-2)).

We used the apparent degree of surface ornamentation to describe the roughness or smoothness of the pollen grain surface.

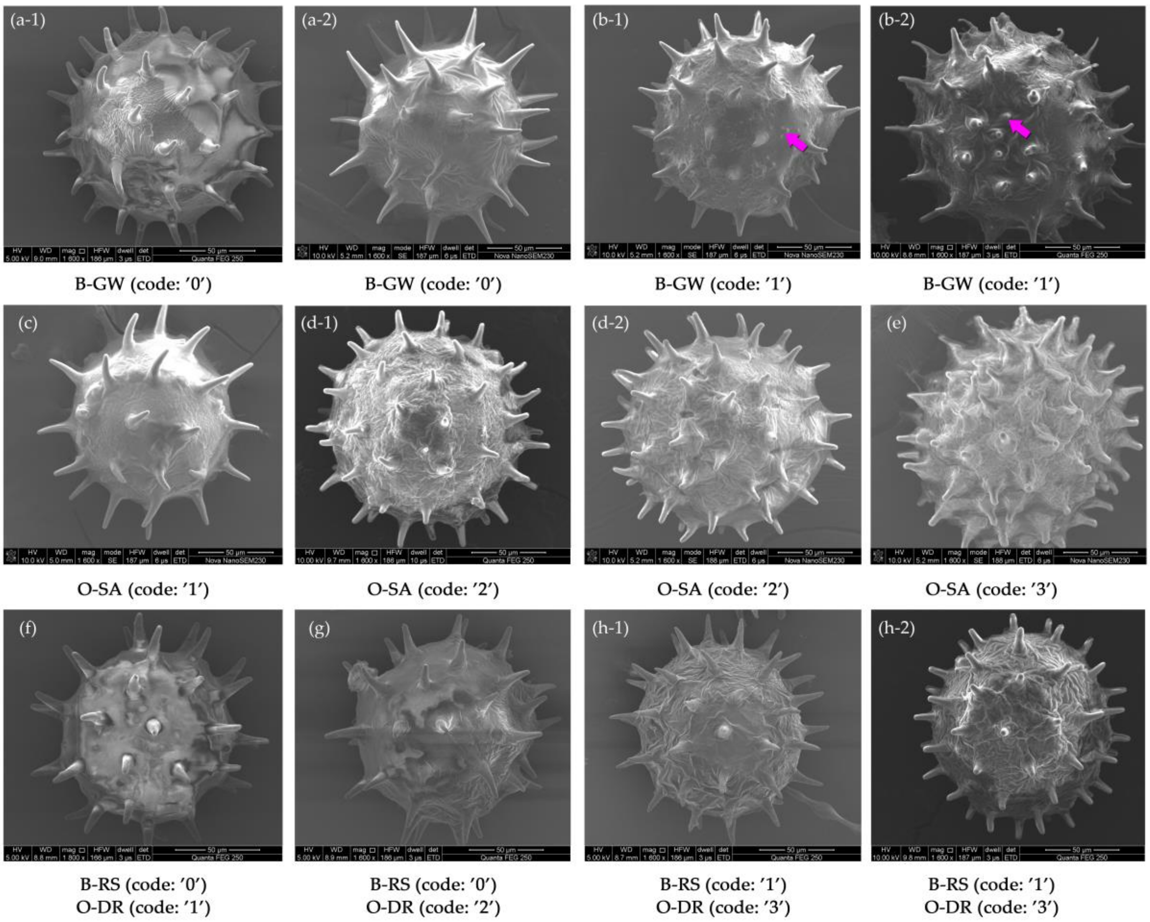

Figure 4. The description and codes for four qualitative traits of *Hibiscus syriacus* pollen grains, where the red arrow indicates the typical characteristic. Whether the pollen surface has granular verrucae (B-GW): (**a-1**,**a-2**) pollen exine is smooth or ruffled without micro-spines or granular verrucae (No, 0); (**b-1**,**b-2**) pollen exine has micro-spines or granular verrucae (Yes, 0). Number of pollen surface spines (O-SA): (**c**) pollen surface spines less than forty in number (<40, 1); (**d-1**,**d-2**) pollen surface spines varying between forty and fifty (40~50, 2); (**e**) pollen surface spines greater than or equal to fifty in number (≥50, 3). Whether the pollen surface ruffles strongly (B-RS): (**f**,**g**) pollen surface not strongly ruffled, smooth, or slightly ruffled (pollen surface ornamentation is inconspicuous or with a small number of ornaments) (No, 0); (**h-1**,**h-2**) pollen surface strongly ruffled (pollen surface with distinctive ornamentation) (Yes, 1). Degree of pollen surface ruffling (O-DR): (**f**) pollen surface smooth and free of ruffles (pollen surface ornamentation is inconspicuous) (smooth and largely unruffled, 1); (**g**) pollen surface is slightly ruffled (pollen surface with a small number of ornaments) (light ruffles, 2); (**h-1**,**h-2**) pollen surface strongly ruffled with striate or a mixture of striate and cerebroid (pollen surface with distinctive ornamentation) (strong ruffles, 3).

3.3. Variation and Correlation of Pollen Morphology between the 24 H. syriacus Cultivars

The range, mean, and cv for eight quantitative traits revealed pronounced differences among the 24 cultivars of *H. syriacus* (Table A1). For example, Chungmu displayed a high level of variation in the traits P and S-spine, while the cultivars Diana, Woodbridge, White chiffon, Qiancenghong, Rubis, and Pyonghwa showed a high level of variation in the traits D_1, D_2/D_1, SL, SW, SL/SW, and D-spine, respectively. Eight quantitative traits were simultaneously tested for a difference among cultivars. This MANOVA result revealed a significant difference among 24 cultivars (Wilk's $\lambda = 0.01$, $F = 7.318$, $p < 0.01$). The follow-up ANOVA results for the eight quantitative traits were as follows: D_1 (F = 8.632), D_2 (F = 8.434), D_2/D_1 (F = 2.913), SL (F = 20.768), SW (F = 12.669), SL/SW (F = 12.228), D-spine (F = 16.262), and S-spine (F = 7.152), which demonstrated there was significant variation among the studied cultivars, as all the eight traits exhibited a high level of statistical significance ($p < 0.01$). According to our post hoc study (Turkey's HSD test) of the 24 cultivars, Paeoniflorus and Naesarang were distinct from the other cultivars by a separate subset of trait A. Other separate subsets were: Pink giant and Woodbridge for trait SL/SW, Pink giant for trait S-spine, and Woodbridge for trait D-spine.

Correlations between pollen quantitative and qualitative traits of *H. syriacus* were also determined. We found more correlations that were significant between quantitative traits than qualitative traits (Figure 5). The trait D-spine was correlated with all six quantitative traits except for SW, which had positive correlations with traits D_1 (0.64, $p < 0.01$) and D_2 (0.46, $p < 0.05$) and negative correlations with traits D_2/D_1 (–0.43, $p < 0.05$), SL (–0.91, $p < 0.01$), SL/SW (–0.82, $p < 0.01$), and S-spine (–0.54, $p < 0.01$). Meanwhile, D-spine showed a significant positive correlation with only one qualitative trait (O-SA, 0.59, $p < 0.01$). Trait P/E had no significant correlation with any traits except for D-spine. Among the qualitative traits, O-SA was correlated with all the quantitative traits except for P/E. In addition, O-DR exhibited a significant positive correlation with B-RS, with both indicators describing the surface ruffling characteristics of *H. syriacus* pollen grains.

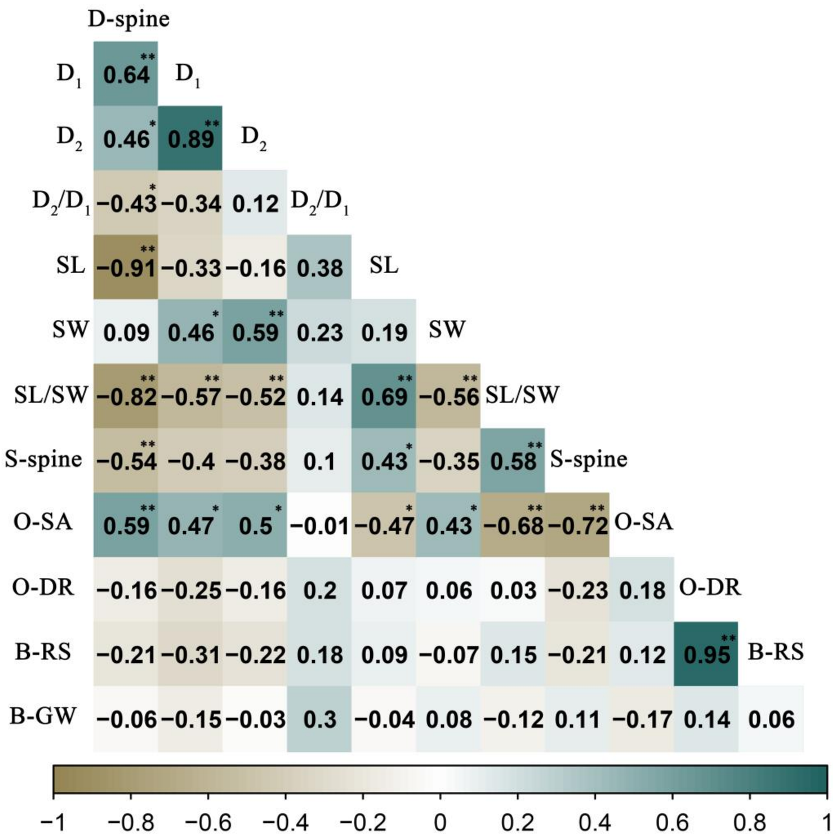

Figure 5. Heatmap of correlations between eight quantitative and four qualitative traits of pollen grains of *Hibiscus syriacus*. The degree of a correlation is indicated by its shaded coloring, with positive or negative numbers in the small square boxes indicating positive or negative correlations between row and column traits, respectively. * $p < 0.05$, ** $p < 0.01$.

3.4. Correlations among Pollen, Flower, and Leaf Morphological Characteristics of *H. syriacus* Cultivars

We tested for correlations between quantitative pollen traits and morphological traits of flowers and leaves of *H. syriacus* (Table 4). The traits D_2, D_2/D_1, SW, and S-spine did not correlate with any flower or leaf traits. Trait E exhibited a significant positive correlation with O-CB (0.550, $p < 0.01$). Trait SL showed positive correlation with both ST (0.496, $p < 0.05$) and B-IP (0.434, $p < 0.05$), but negative correlation with PL (–0.410, $p < 0.05$) and B-LL (–0.478, $p < 0.05$). Trait SL/SM was negatively correlated with PL (–0.565, $p < 0.01$) and O-CB (–0.485, $p < 0.05$), yet positively correlated with B-IP (0.587, $p < 0.01$). Finally, D-spine was positively correlated with three other traits: PL (0.483, $p < 0.05$), O-CB (0.561, $p < 0.01$), and B-LL (0.506, $p < 0.05$), while negatively correlated with B-IP (–0.495, $p < 0.05$).

Table 4. Correlation matrix of pollen vis-à-vis morphological traits of flower and leaf in *Hibiscus syriacus*.

Morphological Traits		Pollen Quantitative Traits							
		D_1	D_2	D_2/D_1	SL	SW	SL/SW	S-Spine	D-Spine
ST	Flower traits	−0.008	0.111	0.247	**0.496** *	0.137	0.265	0.336	−0.387
PL		0.279	0.171	−0.233	**−0.410** *	0.330	**−0.565** **	−0.383	**0.483** *
PW		0.179	0.081	−0.197	−0.080	0.324	−0.292	−0.257	0.173
PL/PW		−0.020	0.002	0.029	−0.342	−0.213	−0.120	0.025	0.246
RC		0.006	0.012	−0.128	0.060	0.379	−0.210	0.049	0.037
RCL		−0.089	−0.151	−0.115	0.094	0.309	−0.149	0.063	−0.053
RCL/RC		−0.033	−0.152	−0.256	0.121	0.077	0.060	−0.014	−0.072
B-IP		−0.301	−0.198	0.237	**0.434** *	−0.302	**0.587** **	0.204	**−0.495** *
B-RC		0.086	0.018	−0.190	0.148	0.105	0.089	−0.004	−0.068
O-CB		**0.550** **	0.381	−0.376	−0.403	0.219	**−0.485** *	−0.194	**0.561** **
O-RC		−0.136	−0.182	−0.038	−0.164	−0.001	−0.177	−0.094	0.123
B-LL	Leaf trait	0.223	0.062	−0.338	**−0.478** *	−0.239	−0.220	−0.110	**0.506** *

Note: Pearson's linear correlation coefficient values between eight pollen quantitative traits and flower and leaf traits. Corresponding details can be found in Tables 2 and 3. The positive or negative numbers in bold indicate positive or negative correlations between row and column traits, respectively. * $p < 0.05$, ** $p < 0.01$.

We also tested for correlations between the 12 pollen traits (Table 2) and the fruiting rates based on data from 11 cultivars of *H. syriacus* (Table A1). These results showed that only B-GW was positively correlated with fruiting rate (0.678, $p < 0.05$). This was an encouraging finding and demonstrated that pollen surface spines and granulates were intrinsically related to pollination.

3.5. Clustering Analysis of 24 H. syriacus Cultivars Based on the 24 Combined Morphological Traits

The PCA results for the 24 *H. syriacus* morphological traits showed a total contribution of 84.55%, in which seven principal components (PCs) were derived. The crucial traits of PC1 (25.81%) were D_2 and D_1; likewise, for PC2 (15.02%), they were SL and D-spine; for PC3 (11.89%), they were RC, RCL, RCL/RC, and B-RC; for PC4 (10.92%), it was PW; for PC5 (10.56%), they were B-RS and O-DR; for PC6 (6.07%), it was O-CB; and for PC7 (4.27%), it was B-GW. PC1, PC2, PC5, and PC7 were indicators related to pollen morphological characteristics, while PC3, PC4, and PC6 were related to flower morphological characteristics.

R-type clustering was used to convey the rationality of indicator selection (Figure 6a). The pairs of traits O-DR and B-RS, RC and RCL, PL and PW, and D_1 and D_2 were clustered at close distances, indicating an equal contribution to the classification of *H. syriacus*. In order to better interpret and compare the effects of traits on the classification, we retained these indicators and used them in a follow-up Q-type clustering analysis.

The results obtained from Q-type clustering revealed that *H. syriacus* cultivars could be divided into 2–6 clusters based on different indicators (Figure 6b):

(1) The 24 cultivars could be divided into two clusters at the grade bond line L1 (D = 20.00) based on the main indicators (D_2, D_1, SW, O-SA, D-spine, and SL). The first cluster included two cultivars (Pink giant and Woodbridge), which presented larger values for the traits D_2, D_1, SW, O-SA, and D-spine, but a smaller value for trait SL. The second cluster comprised the remaining 22 cultivars.

(2) The 24 cultivars could be divided into three clusters at the grade bond line L2 (D = 18.43). The first cluster still consisted of Pink giant and Woodbridge. The remaining 22 cultivars were further classified into two more clusters based on their flower traits (B-RC, RCL, RC, RCL/RC). The second cluster harbored two cultivars (White chiffon and Diana), whose petals lack a red center. The remaining 20 cultivars with the red center constituted the third cluster.

(3) The 24 cultivars could be divided into four clusters at the grade bond line L3 (D = 15.00). In the first cluster was Pink giant and Woodbridge, and in the second cluster was White chiffon and Diana. Then, the other 20 cultivars were further classified into two clusters based on the SL, SW, PW, D-spine, D_2, D_1, O-SA, and O-CB traits. The third cluster had seven cultivars: Pyonghwa, Huaban, Paeoniflorus, Narsarang, Purple pillar, Elegantissimus, and Arang; the fourth cluster entailed the remaining 15 cultivars. The cultivars forming the third cluster presented larger values for SL (17.22~25.04 µm), SW (8.33~12.00 µm), and PW (32.20~50.40 mm), and smaller values for D-spine (2.22~3.32), D_2 (112.39~129.51 µm), and D_1 (111.33~127.13 µm) when compared with those of the fourth cluster. Moreover, the cultivars of the third cluster all had a small number of spines (O-SA, code: 1), and their calyx length was shorter than that of the bract (O-CB, code: 1).

(4) The 24 cultivars were classified into five clusters based on the grade bond line L4 (D = 10.00)—with the same first three clusters for L4 as for L3. The other 15 cultivars were further split into two clusters based on two pollen grain traits (B-RS and O-DR). Two cultivars (Marina and Qiancenghong) were included in the fourth cluster as their pollen grains' surface was weakly and slightly ruffled. The fifth cluster included the remaining 13 cultivars, all of which exhibited strong ruffles.

(5) The 24 cultivars were divided into six clusters based on the grade bond line L5 (D = 7.50). The clustering results at L5 were the same as those at L4, except for the latter's fourth cluster. However, based on two pollen traits (B-RS and O-DR), the fourth cluster at L4 was further divided into two clusters, of which Elegantissimus and Arang grouped together as the fifth cluster as their pollen grains' surface was weakly ruffled. By contrast, the remaining five cultivars were strongly ruffled and thus formed the sixth cluster.

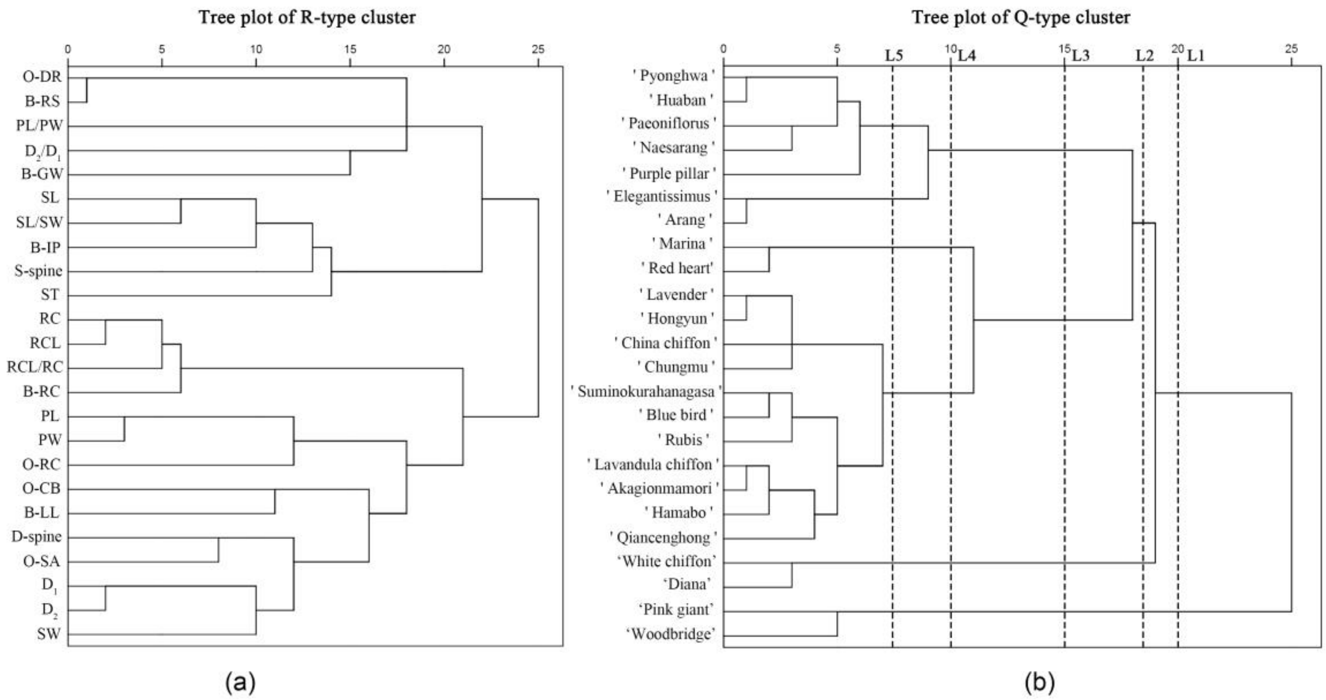

Figure 6. Tree plot of R-type cluster and Q-type cluster of *Hibiscus syriacus*. (**a**) R-type conducted on 24 combined morphological traits based on between-groups linkage method. (**b**) Q-type conducted on *Hibiscus syriacus* 24 cultivars based on ward method.

4. Discussion

4.1. Pollen Morphological Variation of H. syriacus Cultivars and Novel Diagnostic Pollen Traits

Pollen variation diversity can contribute to taxonomic and phylogenetic analyses within the Malvaceae family, whose species can be identified by their spine characteristics [3,29–31]. The pollen of Malvaceae plants is large, being 50~242 μm in diameter, with spines and panpori on the pollen surface, while *Hibiscus* pollen generally varies in size, from 118 to 252.5 μm in diameter [20]. Based on the Palynological Database, *H. syriacus* pollen grains were recorded as a spheroidal shape of large size (>100 μm) [32], and according to Sung et al. and Zhao et al. [3,5], the *H. syriacus* pollen grain diameters ranged from 111.33 to 144.34 μm and 111.65 to 148.98 μm, respectively, while both found high variability in its spine features. In contrast, two indicators of pollen diameter were introduced in this study, D_1 and D_2, with ranges from 111.33 to 144.34 μm and 112.39 to 146.03 μm, respectively, while the ratio of the two indicators (D_2/D_1) described the pollen grain shape. We observed little variation in the pollen shape (D_2/D_1), with that being principally oblate spheroidal (38%) and prolate spheroidal (46%). Four quantitative traits (D_2, D_1, SW, SL, and D-spine) and three new qualitative traits (O-SA, O-DR, and B-RS) examined here could thus be considered as useful diagnostic pollen traits for the classification of *H. syriacus* cultivars.

The trait D–spine takes pollen diameter parallel to the X-axis (D_1) and spine length (SL) into account. Andrade et al. applied this index to distinguish mature pollen grains of *H. rosa-sinensis*, and it was sufficiently accurate to distinguish the species from others in the same family [30]. Here, we employed the D-spine trait for the first time to detect variation between *H. syriacus* cultivars, finding that it made a prominent contribution to the diagnosis and classification of the 24 cultivars, as it showed significant variation among them (range: 2.22~5.38). We suggest the D-spine trait may be an essential pollen trait that can assist in distinguishing species or cultivars of *Hibiscus*. Furthermore, the SW trait, as a new quantitative trait, differed markedly between *H. syriacus* cultivars. At the same time, SW exhibited a positive correlation with D_2, D_1, and O-SA, indicating that cultivars with larger pollen sizes or more spines on their pollen surfaces tend to be accompanied by a broader spine base. The spine index (SL/SW) was also applied here for the first time; however, a weaker contribution to the classification was found for it.

The contribution of qualitative indicators to plant classification can be easily overlooked when they are not quantified. A few qualitative indicators are typically used to describe pollen morphology, usually the pollen surface germinal colpus or porus, and exine ornamentation [20,33]. Spine abundance is often used as a numerical trait for description, as done for *H. syriacus* and *H. rosa-sinensis* [3,30]. Meo et al. found that the number of spine rows between colpi was also the taxonomically important characteristic of *Parthenium hysterophorus* [34]; however, it is weak in its ability to distinguish between plant species or cultivars. Instead, we employed a qualitative trait (O-SA) here to describe the spine abundance, which played a significant role in the classification, with its use as the primary basis for classification at L1 and L3. Furthermore, because the trait O-SA correlated with most quantitative pollen traits, we could assume that pollen grains with large sizes, short spines, or wide spine bases generally also have a higher number of spines. In addition, we employed two new qualitative indicators to describe pollen surface ruffling in two ways. The first is the trait B-RS, for which a smooth or slightly ruffled surface was defined as not strongly ruffled. Most cultivars had a ruffled pollen surface, the five exceptions being Marina, Woodbridge, Elegantissimus, Arang, and Red heart. The second is O-DR, which has three levels of pollen surface ruffling (smooth, lightly ruffled, and extremely ruffled). This trait could further help to distinguish between smooth and slightly ruffled as key features, so that the five cultivars mentioned above could be further clustered into two clusters (one for the first three and one for the last two).

4.2. Combining Morphological Indicators Helps to Better Distinguish Cultivars of H. syriacus and the Contribution of Qualitative Indicators to Clustering

From the results for the Q-type clustering of *H. syriacus* cultivars, the main criteria classification was based on pollen traits at the L1 grade line: D_2, D_1, SW, D-spine, SL, and O-SA. At the L2 grade line, the traits related to the red center of petals (B-RC, RC, RCL, and RCL/RC) enabled us to further classify the remaining 22 cultivars: in this way, two cultivars without the red center were identified (White chiffon and Diana). Prior evidence suggested these (B-RC, RC, RCL, and RCL/RC) indicators could effectively distinguish *H. syriacus* cultivars as PC3 (11.89%) in a classification based on floral morphological traits alone [13]. The present study's results lend further support to using these traits as an independent basis for *H. syriacus* cultivars in a combined classification, in that they were uncorrelated with pollen indicators. At grade line L3, those cultivars with a red center could be further classified into two clusters based on their pollen (SL, SW, D-spine, D_2, D_1, and O-SA) and floral traits (PW and O-CB). Given that O-CB was found positively correlated with E, we may presume those *H. syriacus* cultivars with smaller pollen grains are usually accompanied by a calyx shorter than the bract. Knowledge of this correlation can enable horticulturists to promptly identify *H. syriacus* cultivars, but admittedly more samples are still needed to support this finding. In addition, at grade lines L4 and L5, the qualitative pollen traits related to ruffle features (B-RS and O-DR) were the main classification criteria elucidated. Although the R-type cluster results indicated these two traits are closely related, we kept both since O-DR provided a more in-depth distinction of results generated via B–RS. Lastly, a contribution of the leaf morphological trait (B-LL) to *H. syriacus* classification was not found.

Overall, the results of this study demonstrate that a number of *H. syriacus* cultivars are distinguishable using only quantitative traits. Accordingly, coding and quantifying quantitative traits could contribute to the identification of a greater number of cultivars. Similar results and patterns were reported for *Cathaya argyrophylla* [14]. We propose that the integrated use of morphological indicators of species could generate a broader taxonomic basis.

4.3. Effects of H. syriacus Pollen Traits on Fruiting

Pollen morphological characteristics can influence pollination and breeding, as demonstrated by studies by Mccallum et al., Mendoza et al., and Xia et al., as reported for *Ipomoea purpurea*, *Orius laevigatus*, and *Ottelia acuminata* [27,35,36]. Pollen, seed, and fruit characteristics often have positive correlations [37–39]. The mechanisms by which pollen spines affect fruiting success are complex and challenging to elucidate; they may be related to the spine distribution pattern, spine length, spine density, or even the space distance between spines [17,40]. It would be helpful if we could provide breeding guidance based on the relationships between pollen morphology and pollination or fruit set. We found that the trait B-GW is positively correlated with fecundity (0.678, $p < 0.05$), which indicates that high fruiting rates occur in cultivars that have micro-spine or granular verrucae on their pollen surface; e.g., Blue bird (26.2%) and Red heart (26.0%). Of the 24 studied cultivars, just five harbored this feature (Arang, Rubis, Suminokurahanagasa, Akagionmamori, and Qiancenghong).

Pollen grains with spines and granulate verrucae may adhere to the long hairs of bees for transport, as was previously found for an *Pavonia* sp. (Malvaceae) [41]. However, either pollen size or echinate exine structure alone was not an excellent factor for pollen collectability [40] since the pollination mechanism is complex and related to pollen-collecting bee species, with different genera of bees showing divergence in their collecting behavior [40,42–44]. Fruit set is likely also affected by insect pests [45,46], and whether the incidence of insect pests correlates with pollen morphology or a specific substance warrants further investigation.

Furthermore, floral pollinators' behaviors reflect a selection of pollen traits. Evidence from a study by Lynn et al. of *Taraxacum ceratophorum* revealed that bumblebee pollinators were prone to picking up pollen grains within a narrow distribution of spine distance [17], indicating that a certain spine distance can favor the likelihood of pollination (i.e., a trait selected because it enhances plant fitness vis-à-vis pollinator community). In this respect, *H. syriacus*, a species pollinated chiefly by bumblebees [47], probably has the same selection pattern as described above, and we observed that those cultivars with high fruiting rates also featured a smaller value for spine spacing (S-spine) (Blue bird, 20.78 µm; Red heart, 20.86 µm) than the average for all 24 cultivars (21.14 µm). In addition to pollinators' selection mechanism, which concerns pollen size, spines, and spacing, a pollinator's body size also determines the amount of pollen it can carry, as does its degree of hairiness [16,48]. A model showed that the poor interaction bond between pollenkitt-free spines and pollenkitt-covered exine might weaken the compacting within pollen storage organs; this pattern was prominent on large pollen grains as they reduced the contact surface and thus affected pollen collection [49]. Spine length might also influence pollen adherence to a pollinator's body, but though proven, it is not a significant trait for *Taraxacum ceratophorum* during its pollen pickup [17]. In this study, we obtained an interesting finding that those cultivars with high fruiting rates mainly had smaller values for both E and spine length (SL), e.g., Blue bird and Red heart, which had high fruit sets, whereas their D_1 was 123.36 or 124.52 µm, respectively. By contrast, cultivars with higher D_1 values, namely Pink giant (141.09 µm) and Woodbridge (144.34 µm), had very low fruit sets, at 4.4% and 1.5%, respectively. The traits S-spine and SL did not directly correlate with fruiting rate in our correlation analysis, but they are worth discussing, as effective explanations for this phenomenon are still limited. In this respect, according to the mechanical-defense hypothesis, bumblebees do not collect pollen with bent spines [49]. We observed that some *H. syriacus* cultivars produce pollen with bent spines, but the limited data available in this study prevented us from verifying this hypothesis. Therefore, more *H. syriacus* samples are needed for further testing, to better discern and interpret the relationships between the pollination mechanism and breeding system of *H. syriacus*.

5. Conclusions

Significant variation among studied *H. syriacus* cultivars was demonstrated, especially in their pollen spine features. The derived classification scheme based on flowers and pollen morphological indicators let us classify 24 *H. syriacus* cultivars into 2–6 clusters. The main diagnostic quantitative traits are D_2, D_1, SW, SL, D-spine, RC, RCL, and RCL/RC, while the main diagnostic qualitative traits are O-SA, B-RC, B-RS, O-DR, and O-CB. Among all of those, two new quantitative traits (SW and D-spine) and five new qualitative traits (O-SA, B-RC, B-RS, O-DR, and O-CB) made a robust contribution to the classification of *H. syriacus* cultivars. The number of pollen spines (O-SA) of *H. syriacus* is strongly correlated with its quantitative pollen traits, and three floral (B-IP and O-CB) and leaf (B-LL) phenotypic traits are correlated with certain quantitative pollen traits. The trait B-GW is correlated with fruiting rate, and pollen diameter parallel to the X-axis (D_1), spine length (SL), and spine spacing (S-spine) might all be potential factors that lead to successful breeding in *H. syriacus*.

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Institutional Review Board Statement: The study did not require ethical approval.

Informed Consent Statement: The study did not involve humans.

Data Availability Statement: Data available from the author.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The minimal, maximal, mean values and coefficient of variation (cv) for eight quantitative pollen traits of *Hibiscus syriacus*.

Samples	D_1			D_2			P/E			SL		
	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv
1	130.70	110.89–145.87	7.67%	124.04	104.11–131.79	6.47%	0.95	0.82–1.13	8.75%	15.07	13.32–17.91	8.94%
2	123.03	110.35–136.40	9.52%	131.06	120.92–139.25	5.04%	1.07	0.93–1.18	7.50%	16.66	13.14–22.76	17.09%
3	141.09	132.97–145.58	2.45%	146.03	132.60–158.22	6.32%	1.03	0.97–1.13	4.91%	15.65	13.25–17.80	10.45%
4	127.95	122.82–134.50	3.31%	129.78	123.55–135.09	2.88%	1.01	1.00–1.04	1.06%	16.81	12.48–20.26	14.83%
5	127.13	119.37–133.47	3.60%	129.30	110.46–138.07	6.04%	1.02	0.85–1.06	6.31%	23.70	20.15–27.10	8.82%
6	144.34	133.46–160.95	5.64%	139.12	112.37–148.10	9.58%	0.97	0.73–1.05	13.09%	13.42	12.09–15.49	8.25%
7	120.83	98.91–135.14	13.77%	117.76	103.68–128.76	9.53%	0.98	0.93–1.05	4.77%	15.31	13.84–17.55	8.02%
8	123.44	112.96–140.53	8.66%	121.44	116.96–126.63	3.09%	0.99	0.90–1.04	5.43%	15.55	12.73–19.05	12.33%
9	126.29	119.89–129.83	2.88%	125.44	120.65–129.62	2.11%	0.99	0.97–1.03	1.90%	19.56	16.64–22.36	9.50%
10	125.19	105.58–133.40	9.44%	129.51	105.82–145.29	10.70%	1.03	0.99–1.10	3.71%	20.15	15.08–23.40	11.81%
11	119.08	90.19–131.66	10.38%	123.24	105.56–138.39	9.84%	1.04	0.99–1.17	5.21%	19.73	17.73–21.13	4.98%
12	118.66	112.55–125.56	4.22%	118.32	104.18–127.02	5.91%	1.00	0.92–1.07	5.27%	16.73	14.02–19.65	11.38%
13	114.59	104.39–128.85	5.31%	119.76	111.69–129.57	4.43%	1.05	0.97–1.15	5.04%	19.33	14.68–23.21	13.45%
14	123.16	113.81–135.99	5.95%	126.94	119.62–134.17	4.04%	1.03	0.96–1.10	4.17%	14.45	11.50–18.30	15.58%
15	122.76	90.72–137.44	12.08%	119.16	90.9–135.96	12.10%	0.97	0.90–1.05	4.50%	14.20	11.32–17.95	14.25%
16	116.54	109.08–127.62	5.93%	119.18	111.99–127.62	4.35%	1.02	0.92–1.12	5.35%	15.06	12.14–19.45	16.80%
17	117.64	108.77–128.85	4.93%	116.95	113.16–121.18	1.99%	1.00	0.91–1.06	4.53%	20.24	13.58–24.71	15.39%
18	121.06	114.61–130.31	3.80%	123.36	113.45–138.66	4.15%	1.02	0.95–1.10	5.40%	16.13	12.71–19.12	13.25%
19	126.71	102.54–144.72	8.79%	126.80	103.63–131.22	7.64%	1.00	0.95–1.04	2.75%	18.54	17.05–22.53	8.84%
20	125.33	117.65–138.44	5.55%	124.52	111.99–137.85	5.34%	0.99	0.94–1.04	3.75%	16.08	10.73–19.42	15.35%
21	138.20	132.71–143.62	2.54%	134.37	131.24–138.45	1.80%	0.97	0.94–1.00	2.00%	18.47	16.41–20.07	4.99%
22	121.18	103.63–125.44	5.26%	121.79	99.99–138.45	7.65%	1.00	0.96–1.11	4.22%	14.54	12.00–19.55	16.41%
23	114.31	102.90–123.26	5.79%	112.39	102.9–118.68	5.38%	0.98	0.93–1.02	3.45%	17.22	14.38–18.61	8.40%
24	111.33	98.55–120.09	7.61%	116.22	97.27–126.29	9.04%	1.04	0.99–1.10	3.58%	25.04	22.89–28.24	6.78%

Samples	SW			SL/SW			D–spine			S–spine		
	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv	Mean	Range	cv
1	8.59	6.61–10.15	13.85%	1.79	1.31–2.29	18.30%	4.36	3.50–5.01	10.76%	20.36	16.26–23.51	11.20%
2	9.01	7.13–10.67	12.35%	1.85	1.53–2.37	11.96%	3.77	2.93–5.19	17.18%	22.09	16.52–27.74	16.03%
3	15.00	10.99–18.61	16.54%	1.06	0.90–1.37	16.21%	4.56	3.78–5.43	11.45%	12.85	9.98–19.73	20.57%
4	8.37	6.73–9.86	10.84%	2.01	1.78–2.42	8.71%	3.88	3.12–4.99	15.57%	19.19	14.80–22.74	12.66%
5	10.43	8.99–12.14	10.53%	2.29	1.97–2.61	10.20%	2.70	2.23–3.24	10.59%	20.49	16.14–26.44	16.78%
6	12.73	11.54–14.36	6.43%	1.06	0.84–1.28	11.54%	5.40	4.72–6.00	7.56%	18.75	16.20–23.38	12.21%
7	9.47	8.13–11.56	11.07%	1.63	1.36–1.83	9.18%	3.96	3.33–4.83	13.62%	20.28	14.57–27.85	18.96%
8	8.96	6.94–10.51	12.32%	1.75	1.57–2.11	11.10%	4.00	3.62–4.62	8.81%	18.98	15.15–22.57	15.51%
9	9.93	8.12–12.70	17.00%	2.02	1.38–2.63	18.93%	3.26	2.82–3.76	10.69%	25.63	20.22–31.54	14.08%
10	9.66	8.73–11.28	8.16%	2.09	1.64–2.36	9.77%	3.15	2.48–4.38	17.25%	27.37	20.57–32.75	13.79%
11	10.66	9.32–11.72	7.34%	1.86	1.60–2.12	8.49%	3.03	2.23–3.46	12.69%	24.14	15.73–30.56	19.24%
12	9.18	6.57–11.33	14.99%	1.84	1.40–2.22	12.72%	3.59	2.93–4.26	11.79%	18.04	11.51–21.48	16.09%
13	12.00	9.19–14.48	13.54%	1.66	1.01–2.24	23.53%	3.01	2.41–3.56	12.51%	23.74	16.28–30.04	20.59%
14	9.64	6.50–12.36	19.02%	1.56	0.93–2.42	28.34%	4.36	3.17–5.65	17.97%	18.57	14.37–26.65	21.80%
15	9.24	7.28–12.56	15.69%	1.56	1.21–1.88	15.62%	4.44	2.86–5.85	22.93%	22.70	11.66–29.09	22.96%
16	8.25	7.27–10.35	10.88%	1.84	1.43–2.32	16.82%	3.97	3.01–5.26	18.22%	21.90	17.93–28.00	17.12%
17	9.19	7.25–11.59	15.83%	2.22	1.87–2.72	13.02%	3.00	2.20–4.74	23.33%	24.65	18.59–31.32	15.44%
18	8.74	5.31–11.31	22.37%	1.91	1.40–2.51	20.75%	3.81	3.08–4.75	13.89%	20.78	17.90–28.74	17.10%
19	8.50	7.02–9.84	9.72%	2.19	1.93–2.53	8.75%	3.44	2.68–3.87	11.56%	23.45	20.04–27.43	10.08%
20	8.48	4.91–10.04	17.15%	1.91	1.70–2.19	8.55%	3.99	3.26–5.74	17.63%	20.86	18.13–23.08	8.60%
21	10.88	6.92–14.54	22.89%	1.78	1.38–2.64	23.71%	3.75	3.46–4.30	5.91%	20.92	14.03–27.63	21.24%
22	8.43	6.57–10.94	16.13%	1.73	1.50–2.05	10.94%	4.26	3.20–5.13	16.02%	20.23	15.00–24.85	14.00%
23	8.34	6.50–10.60	17.78%	2.11	1.67–2.58	14.32%	3.34	3.00–4.06	8.87%	20.40	18.57–23.90	7.57%
24	11.59	9.15–14.28	17.70%	2.23	1.76–3.09	21.53%	2.23	1.92–2.48	8.93%	21.11	14.46–28.10	17.76%

Table A2. The fruiting rates of 11 *Hibiscus syriacus* cultivars.

No.	Cultivar	Fruiting Rate (%)
1	'Pink giant'	4.4
2	'Woodbridge'	1.5
3	'Elegantissimus'	0.1
4	'Arang'	15.8
5	'Rubis'	13.2
6	'Chungmu'	0.1
7	'Suminokurahanagasa'	2.3
8	'Blue bird'	26.2
9	'Akagionmamori'	0.1
10	'Red heart'	26.0
11	'Naesarang'	1.0

References

1. Liu, Z.; Gao, L. Ancient *Hibiscus syriacus* culture of China. *Agric. Archaeol.* **2010**, *4*, 228–231.
2. National Institute of Forest Science. *Illustration of Hibiscus syriacus L. cultivars*; Seoul Press: Seoul, Republic of Korea, 2014.
3. Bae, S.H.; Younis, A.; Hwang, Y.J.; Lim, K.B. Various Pollen Morphology in *Hibiscus syriacus*. *Flower Res. J.* **2015**, *23*, 125–130. [CrossRef]
4. He, L. Comparision in Cytology and Molecular Biology of Simple and Double Flower on *Hibiscus syriacus*. Ph.D. Thesis, Beijing Forestry University, Beijing, China, 2006.
5. Zhao, Y.; Feng, Q.; Tian, L.; Zhang, J.; Wang, X.; Liu, Y.; Liu, D. Pollen morphology and numerical taxonomy of 22 *Hibiscus syriacus*. *Guihaia* **2021**, *41*, 103–113. [CrossRef]
6. Oliveira, P.P.; dos Santos, F.d.A.R. Morfologia polínica de Hibiscus pernambucensis Arruda e *Hibiscus tiliaceus* L. (Malvaceae). *Acta Biol. Leopoldensia* **2004**, *26*, 203–211.
7. EI, N.S. Pollen morphology of Egyptian Malvaceae: An assessment of taxonomic value. *Turk. J. Bot.* **2004**, *28*, 227–240.
8. Yuan, T.; Wang, L. Pollen Morphology of Several Tree Peony Wild Species and Discussion on Its Evolution and Taxonomy. *J. Beijing For. Univ.* **1999**, *21*, 18–21.
9. Palazzesi, L.; Pujana, R.R.; Burrieza, H.P.; Steinhardt, A.P. Pollen grain morphology of selected allergenic species native to Southern South America. *J. Torrey Botanic. Soc.* **2007**, *134*, 527–533. [CrossRef]
10. Korszun, S.; Klimko, M. Microsporangia and pollen morphology of Ginkgo biloba cultivars. *Dendrobiology* **2014**, *71*, 83–92. [CrossRef]
11. Ma, S.L.; Lu, Y.M. Classification and phylogenetic analysis of Chinese hawthorn assessed by plant and pollen morphology. *Genet. Mol. Res. Gmr* **2016**, *15*, gmr.15038739. [CrossRef] [PubMed]
12. Ma, Z.H.; Glc, B.; Zhang, D.X. Pollen morphology of Callicarpa L. (Lamiaceae) from China and its systematic implications. *Plant Syst. Evol.* **2016**, *302*, 67–88. [CrossRef]
13. Xiao, F.; Wang, X.H.; Wang, Y.Q.; Liu, C.L.; Xie, L.S.; Ren, X.Y. Numerical Classification and Principle Component Analysis of 27 Hibiscus Cultivars. *J. Cent. South Univ. For. Technol.* **2019**, *39*, 59–64.
14. Xiao, F.; She, Y.; She, J.; Wang, Y.; Wu, F.; Xie, P.; Chen, Q. Intraspecific Pollen Morphology Variation and Its Responses to Environmental Factors of Wild *Cathaya argyrophylla* Chun Et Kuang Endemic to China. *Forests* **2022**, *13*, 651. [CrossRef]
15. Minnaar, C.; Anderson, B.; de Jager, M.L.; Karron, J.D. Plant–pollinator interactions along the pathway to paternity. *Ann. Bot.* **2018**, *123*, 225–245. [CrossRef] [PubMed]
16. Phillips, B.B.; Williams, A.; Osborne, J.L.; Shaw, R.F. Shared traits make flies and bees effective pollinators of oilseed rape (*Brassica napus* L.). *Basic Appl. Ecol.* **2018**, *32*, 66–76. [CrossRef]
17. Lynn, A.; Piotter, E.; Harrison, E.; Galen, C. Sexual and natural selection on pollen morphology in Taraxacum. *Am. J. Bot.* **2020**, *107*, 364–374. [CrossRef]
18. Xu, K.X. *Numerical Taxonomy*; Science Publishing: Beijing, China, 1994.
19. Erdtman, G.; Vishnu-Mittre. On Terminology in Pollen and Spore Morphology. *Grana Palynol.* **1958**, *1*, 6–9. [CrossRef]
20. Wang, F.X.; Qian, N.F.; Zhang, Y.L.; Yang, H.Q. *Pollen Flora of China*, 2nd ed.; Science Publishing: Beijing, China, 1995; pp. 5–13.
21. Punt, W.; Hoen, P.P.; Blackmore, S.; Nilsson, S.; Le Thomas, A. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.* **2007**, *143*, 1–81. [CrossRef]
22. Erdtman, G. The Acetolysis Method. A Revised Description. *Sven. Bot. Tidskr.* **1960**, *54*, 561–564.
23. Abdi, H.; Williams, L.J. Principal Component Analysis. *WIREs Comput. Stat.* **2010**, *2*, 433–459. [CrossRef]
24. Martinović, S.; Vlahović, M.; Gajić-Kvaščev, M.; Vuksanović, M.; Glišić, D.; Volkov-Husović, T. Principal component analysis of morphological descriptors for monitoring surface defects induced by thermal shock. *J. Eur. Ceram. Soc.* **2021**, *41*, 426–429. [CrossRef]
25. Fukunaga, K. *Introduction to Statistical Pattern Recognition*; Academic Press: Cambridge, MA, USA, 1990. [CrossRef]
26. Parks, J.M. Part 2: Applications of Multivariate Statistics in Geology | |Cluster Analysis Applied to Multivariate Geologic Problems. *J. Geol.* **1966**, *74*, 703–715. [CrossRef]
27. Mccallum, B.; Chang, S.M. Pollen competition in style: Effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. *Am. J. Bot.* **2016**, *103*, 466–468. [CrossRef] [PubMed]
28. Xiao, F.; She, Y.; She, J.; Zhang, J.; Zhang, X.; Luo, C. Assessing habitat suitability and selecting optimal habitats for relict tree Cathaya argyrophylla in Hunan, China: Integrating pollen size, environmental factors, and niche modeling for conservation. *Ecol. Indic.* **2022**, *145*, 109669. [CrossRef]
29. EI, N.S.; Sawady, N. Pollen morphology of Malvaceae and its taxonomic significance in Yemen. *Flora Medit* **2008**, *18*, 431–439.
30. Andrade, K.; Guerra, S.; Debut, A. Fullerene-Based Symmetry in Hibiscus rosa-sinensis Pollen. *PLoS ONE* **2014**, *9*, e102123. [CrossRef]
31. Shaheen, N.; Khan, M.A.; Hayat, M.Q.; Yasmin, G. Pollen morphology of 14 species of Abutilon and Hibiscus of the family Malvaceae (sensu stricto). *J. Med. Plants Res.* **2009**, *3*, 921–929.
32. Halbritter, H.; Heigl, H. *Hibiscus syriacus*. In *PalDat—A Palynological Database*. Available online: https://www.paldat.org/pub/Hibiscus_syriacus/304207 (accessed on 7 March 2023).
33. Hao, M.; Cao, F.; Zhang, W.; Wang, G. Variation of pollen morphology among male trees of Ginkgo. *J. For. Eng.* **2006**, *20*, 53–55.

34. Meo, A.A.; Khan, M.A. Pollen morphology of invasive species *Parthenium hysterophorus* L. (Heliantheae—Asteracea) from Islamabad and Rawalpindi, Pakistan. *J. Agric.* **2005**, *21*, 227–230.
35. Mendoza, J.E.; Balanza, V.; Cifuentes, D.; Bielza, P. Selection for larger body size in *Orius laevigatus*: Intraspecific variability and effects on reproductive parameters. *Biol. Control* **2020**, *148*, 104310. [CrossRef]
36. Xia, J.; Lu, J.; Wang, Z.; Hao, B.; Wang, H.; Liu, G. Pollen limitation and Allee effect related to population size and sex ratio in the endangered *Ottelia acuminata* (Hydrocharitaceae): Implications for conservation and reintroduction. *Plant Biol.* **2013**, *15*, 376–383. [CrossRef]
37. Satil, F.; Kaya, A.; Ünal, M. Fruit, seed and pollen morphology of Chorispora DC. Species (Brassicaceae) of Turkey. *Bangladesh J. Bot.* **2018**, *47*, 459–466. [CrossRef]
38. Doi, K.; Inoue, R.; Iwasaki, N. Seed weight mediates effects of pollen on berry weight, ripening, and anthocyanin content in highbush blueberry. *Sci. Hortic.* **2021**, *288*, 110313. [CrossRef]
39. Maryam; Jaskani, M.J.; Ahmad, S.; Awan, F.S. Metaxenial Effects on Morphological Attributes in Date Palm cvs. Hillawi and Khadrawy. *Pak. J. Agric. Sci.* **2015**, *52*, 387–393.
40. Konzmann, S.; Koethe, S.; Lunau, K. Pollen grain morphology is not exclusively responsible for pollen collectability in bumble bees. *Sci. Rep.* **2019**, *9*, 4705. [CrossRef] [PubMed]
41. Schlindwein, C.; Pick, R.A.; Martins, C.F. Evaluation of oligolecty in the Brazilian bee *Ptilothrix plumata* (Hymenoptera, Apidae, Emphorini). *Apidologie* **2009**, *40*, 106–116. [CrossRef]
42. Michener, C.D. The bees of the world. In *Systematic Entomology*; J.H.U. Press: Baltimore, MD, USA, 2010; Volume 26, pp. 255–256.
43. Aguilar, C.; Azucena, M.; Parra-Tabla, V. Importance of conserving alternative pollinators: Assessing the pollination efficiency of the squash bee, *Peponapis limitaris* in *Cucurbita moschata* (Cucurbitaceae). *J. Insect Conserv.* **2000**, *4*, 201–208. [CrossRef]
44. Ballantyne, G.A.; Baldock, K.; Willmer, P.G. Constructing more informative plant-pollinator networks: Visitation and pollen deposition networks in a heathland plant community. *Proc. R. Soc. Biol. Sci.* **2015**, *282*, 20151130. [CrossRef]
45. Jung, J.-K.; Kim, M.; Lee, C.Y.; Jang, B.-J.; Kim, D.; Kwon, H.Y.; Park, Y. Comparison of Insect Pest Communities on 30 Cultivars of *Hibiscus syriacus*. *J. Korean Soc. For. Sci.* **2021**, *110*, 116–127.
46. Kim, Y.; Cho, Y.; Kang, Y.K.; Choi, M.; Nam, S.H. A study of the major insect pest communities associated with *Hibiscus syriacus* (Columniferae, Malvaceae). *J. Ecol. Environ.* **2013**, *36*, 125–129. [CrossRef]
47. You, X.; Zhang, D.; Chi, J.; Wu, H. ComparatiVe study on three *Hibiscus syriacus* cultivars' characteristics of nectar secretion, and pollen presentation and entomophilous pollination. *Acta Agric. Shanghai* **2009**, *25*, 81–86.
48. Stavert, J.R.; Linan-Cembrano, G.; Beggs, J.R.; Howlett, B.G.; Pattemore, D.E.; Bartomeus, I. Hairiness: The missing link between pollinators and pollination. *Peerj* **2016**, *4*, e2779. [CrossRef] [PubMed]
49. Lunau, K.; Piorek, V.; Krohn, O.; Pacini, E. Just spines—Mechanical defense of malvaceous pollen against collection by corbiculate bees. *Apidologie* **2015**, *46*, 144–149. [CrossRef]