Morphological Variation In Pollen Grains Of Philippine Hibiscus Rosa-Sinensis Hybrids

Divine Joy A. Mauhay, Larry V. Padilla, Fe Corazon A. Jacinto, Eileen Z. Vitug

Abstract: Hybridization of both plants and animals has innumerably benefitted man. An example of which is the numerous hybrids of Hibiscus rosa-sinensis which are primarily used for aesthetic purposes because of their colourful flowers. Phenotypic variations can already be observed in various parts of H.rosa-sinensis because of hybridization; hence, it is likely that modifications are occurring on microscopic structures such as the pollen. Through time, such variations could change the frequencies of alleles in the gene pool and could possibly lead to microevolution of the species. This study focused on the determination of variations in pollen grain morphology of ten (10) selected H. rosa-sinensis hybrids from the Institute of Plant Breeding of the University of the Philippines-Los Banos, specifically in terms of pollen aperture, size, shape, length of spine and sculpturing. The pollen shape, type of aperture and sculpturing were determined qualitatively. One-way ANOVA was employed if there is significant difference among the pollen of the hybrids in terms of the said quantitative characters. Pollen shape variation was determined through Elliptic Fourier Coefficient Analysis. Results showed that all hybrids have pantoporate type of aperture, echinate type of sculpturing, and spheroidal shape. Among the characters observed, variation was noted in their pollen size and spine length. Pollen size ranges from large to very large and long to very long spine length. Majority of the hybrids observed (7 out of 10) have very large pollen size and long pollen spines. One (1) hybrid has very large pollen size and short spines while two (2) have large pollen size and short pollen spines. There was also significant difference among the samples in terms of these characters based on statistical analysis. The hybrids with variations (Claire Baltazar x Cely Hermosa, Diamond Star and Vicky) cannot be considered outgroups on the basis of the said quantitative characters alone. Nevertheless, such variations observed should not be discounted as a possible modification in pollen morphology in progress as a result of hybridization.

Index Terms: Hibiscus, hybridization, diagnostic character, outgroup, pollen morphology, pollen grains, variation

1. INTRODUCTION

Pollen grains are vegetative cells of both angiosperms and gymnosperms that contain male gamete. It carries the male DNA to the female part of the plant. Thus, it must be strong enough to protect the DNA on its journey, which is evident in its structure. It has an outer wall, called exine composed of multilayered very tough polymer called sporopollenin, which imparts resistance against degradation by chemicals, bacteria and fungi. It specifically protects the sperm nucleus from desiccation and irradiation during transport from the anther to stigma. Its inner layer, the intine, is also sometimes multilayered and is primarily composed of cellulose. It encloses the cytoplasm and the organelles. The pollen coat is composed of lipids, proteins, pigments and aromatic compounds that fill the cavities of the exine. Aside from reproduction, pollen also functions as reward to the plant's visitors [1]. Palynology has been used in plant phylogenetic analysis and identification of both extant and fossil plants (e.g. paleopalynology, paleobotany). This is based on the assertion that every type of pollen has its exclusive characteristics [1]. In a study by Angulo et al. [2], they investigated the systematic value of micromorphological and palynological characters in Stenocephalum wherein said features were noted valuable within genera to differentiate related species from one another. Thus, the morphological features of pollen form basis for the identification and characterization of a particular taxon.

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Some of these features which categorize and distinguish one type of pollen to another are size, shape, symmetry, aperture, sculpturing, polarity, unit and storage. These patterns are specific for a certain species and are established early during cell division. Hybridization has been a significant tool, which allowed man to prosper. An example is the hybridization of Bos Taurus (European domestic cattle) with B. indicus (Zebu). This produced several breeds including Beefmaster (Zebu x Shorthorn) and Brangus (Zebu x Angus). In plants, there are different hybrids of Mussaenda philippica var.aurorae Sulit, more popularly known as "Dona Aurora" [3], [4]. From the crosses and backcrosses of Dona Aurora with M. philippica, 15 immediate hybrids were produced such as "Doña Esperanza", "Doña Luz", "Doña Pacencia", to name a few. They basically differ in leaf shape, flower color, and pollen fertility of the hybrids. Another popular plant being hybridized is the Hibiscus rosa-sinensis. Hibiscus rosa-sinensis belong to family Malvaceae or mallow family [5]. It is believed that the Chinese traders were the ones who introduced Hibiscus in the Philippines [6]. H. rosa-sinensis has become popular as an ornamental plant. To date, new varieties are being developed by cross breeding. Hibiscus breeding program in the Philippines started in 1994 by Mr. Reynold Pimentel at the Institute of Plant Breeding of the University of Philippines-Los Baños then continued by Dr. Pablito Magdalita in 2002 to present [7]. From the hybridization of local cultivars with foreign varieties, several "series" or batches of hybrids were produced that were named after some Filipina (e.g. Millennium Series, Women in Science Series, Celebrity Stars Series, Oblation Series, Women in Public Series, Women in the Arts Series). For instance, the Centennial Series is composed of 11 hybrids named after the 11 Filipino heroines who fought for the country's freedom against Spaniards and Americans. Like in Mussaenda hybrids, Hibiscus hybrids differ in leaf shape, flower size, flower color, etc [8]. It can be noted that they still belong to the same species applying the biological species concept despite their phenotypic variations. Given such variations, a diagnostic character could possibly be present wherein such character could be used to distinguish an

individual from all others [9]. This study investigated if variation is occurring in terms of pollen features among the selected H. rosa-sinensis hybrids and attempted to identify which among the characters serves as a diagnostic character for identification and classification. The findings may contribute to the study of systematics of angiosperms, specifically the H. rosa-sinensis hybrids.

2. LITERATURE REVIEW

Morphological characters are commonly used as diagnostic characters since they are readily observable features. In plants, some of the diagnostic characters are leaves (ex. phyllotaxy, venation, etc.), flowers (e.g. type of inflorescence, perianth, etc.), type of fruit, position of ovary, type of habit, and others. The study of Mantovani, et al [10] suggested that the midrib outline in cross section has a high potential for the diagnosis of Anthurium section Urospadix subsection Flavescenti viridia. Several studies have shown the significance of palynology in systematics and taxonomy. One of which is the study of Perveen [11] wherein he cited that palynology can provide remarkable information due to some pollen features such as the sculpturing of its exine that makes it a diagnostic character. He examined 67 families of angiosperms and the results revealed great pollen diversity in terms of their qualitative characters. He also mentioned that polarity, symmetry, apertural types and exine sculpturing are the important characters from a phylogenetic and evolutionary point of view. Angulo et al. [2] observed highly significant differences in pollen features among the species of Stenocephalum, and combined with the different micromorphological features, the data were important to differentiate related species from one another. In the same note, Mostafa et al. [12] studied the pollen morphology of four genera under family Dipsacaceae as possible taxonomic characters. Their results showed the transfer of some Scabisoa species to Lomelosia Raf. based on palynological characters. Their study also mentioned that pollen characteristics have been used to several disputed genera and problems related to the origin and evolution of many taxa, as well as classification of angiosperms. Moreover, findings of the study of Thornhill et al. [13] indicate the possible use of the morphology of colpus in diagnosing pollen types in Myrtaceae. On the other hand, Christensen [5] studied the pollen morphology of Malvaceae and compared the obtained results with recent classifications of the study. The characters that he observed include pollen size, aperture, shape, spine, and sculpturing. The colorful hybrids of Mussaenda that are commonly grown locally are from Mussaenda philippica var aurorae Sulit [3]. It was discovered at the forest reserve under the University of the Philippines Los Banos in 1915. This was successfully propagated asexually and was locally named as “Dona Aurora” in 1938 after the wife of President Manuel L. Quezon, Mrs. Aurora Quezon. Several cultivars have been developed at the University of the Philippines Los Banos [4]. Some of them are named after the First Ladies of the Philippines (e.g. Dona Evangelina, Dona Luz, Dona, Imelda, etc.). Other mussaendas are named ‘Mutya’, ‘Diwata’, ‘Paraluman’ and ‘Bathaluman’, which are synonymous to “muse”. Those names are titles given to Filipino ladies chosen for their poise, grace, beauty and intelligence. The cultivars differ morphologically. For instance, the cultivar M. ‘Gloria-Macapagal-Arroyo’ is a cross between M. ‘Dona Evangelina (with red bracts) and M. ‘Dona Aurora” (with white bracts). The resulting offspring has bracts that are 4.0 x 1.7 – 4.0 x 2.1 cm, oblong to ovate elliptic and basically white with tiny rose areas and with claret rose margin while the flowers are orpiment orange, 1.5-1.8 cm across and sterile. On the other hand, M. ‘Zenaida Umali’ (M. ‘Dona Aurora’ x M. ‘Paraluman’) has 6.5 x 4.0 – 8.0 x 4.5 cm creamy and obovate bracts. The flowers are sulfur yellow, 2.2-2.4 cm across and semi-fertile. Similar to Mussaenda, differences in morphology exist in the cultivars of Hibiscus rosa-sinensis. In the study of Cabarrubias, et al. [8], 57 hybrid progenies of Hibiscus from different crosses were evaluated and characterized for morphological traits for selection of hybrids with unique color and form. One of the results showed that the hybrids with large flowers and longer petioles tend to have wider leaves. In another study, Guerra et al. [14] analyzed the pollen morphology of H. rosa-sinensis in three stages (bud, anthesis and senescence). The parameters used were spine index, D-spine index, and spine density. However, based on the results of the study, the spine index factor that they used was not sufficient for taxonomic classification of Hibiscus, mainly due to the evolution of such factor in the three studied stages.

3. METHODOLOGY

Ten (10)Hibiscus rosa-sinensis hybrids from the Institute of Plant Breeding of the University of the Philippines Los Banos were examined in this study: (1) Claire Baltazar x Cely Hermosa, (2) Cheery twirl, (3) Aislyn, (4) LorenLegarda x Estrella Alabastro, (5)Gelia Castillo x Diamond Star, (6) Cheche Lazaro, (7) Vicky, (8) Ningning, (9) Diamond Star, and (10) Evelyn Mae Mendoza (Fig 1).

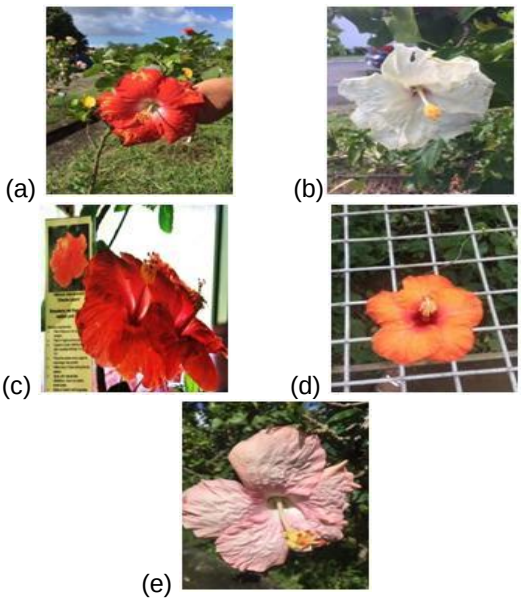

Fig 1. *Some selected H. rosa-sinensis hybrids(a) Cheery Twirl; (b) Diamond Star; (c) Cheche Lazaro; (d) Vicky; (e) Loren Legarda x Estrella Alabastro;*

Pollen grains were obtained from the anther of each flower. The collected pollen grains were processed and subjected to pollen analysis based on the procedure for acetolysis proposed by the International Commission for Bee Botany [15] with some modifications. Acetolysis solution was composed of 9 parts glacial acetic acid and 1 part HCl. Safranin O and glycerine were added for clearer visualization of the pollen and as mountant, respectively. From each flower sample, 6 pollen

grains were randomly observed. Among the palynological features, the aperture, size, shape, length of spine and sculpturing were observed and analyzed using a photomicroscope with a total magnification of 400x. The specific types of each pollen feature were used as basis for classification [16]. Pollen size categorization was based on Erdtman's classification. Pollen size and length of spine were measured using calibrated ocular micrometer. One-way ANOVA was employed to determine if there is a significant difference among the samples in terms of pollen size and pollen spine length (α = 0.05). Pollen shape variation was determined through Elliptic Fourier Coefficient Analysis using Shape 1.3software [17]. Classification of spine length was arbitrarily designated in the present study. Pollen were considered "short" if spine length measures 1-15 µm while 'long' if 16-30 µm long

4. RESULTS AND DISCUSSION

The following presents the results and discussion of the observation and analysis of the morphological characteristics of the pollen of selected H.rosa-sinensis hybrids, specifically, aperture, size, shape, length of spine and sculpturing. Figure 2 shows the representative pollen from each hybrid. Most share similar pollen traits with variation on size and length of spine. Based on pollen size measurement, most can be classified as 'very large' except for Diamond Star and Vicky hybrids, which were characterized as 'large' (Table 2). Cheche Lazaro has the largest average pollen size 148.8µm, followed by Gelia Castillo x Diamond Star (139.2 µm) and Claire Baltazar x Cely Hermosa (138.3 µm). Vicky hybrid was noted to have smallest average size (85.4µm). Moreover, there was a significant difference ($p<0.001$ α 0.05 level) in the pollen size among the samples based on One-Way ANOVA. The pollen size obtained in this study is similar to the study of Bibi et al. [18] wherein they classified Hibiscus pollen as 'very large' (about 165 µm). In addition, members of Malvaceae have pollen grain size of 150-200 µm. However, size is regarded by many as the least reliable morphological feature of pollen grains due to possible variation with age and laboratory methods [19]. On the other hand, the idea that hybridization could have effect on pollen morphology such as size is also suggestive.In terms of pollen spine, majority of the hybrids studied have long spines, while three (3) have short spines namely Claire Baltazar x Cely Hermosa, Diamond Star, and Vicky (Table 3). Loren Legarda x Estrella Alabastro and Cheery twirl both have a long spines with a mean length of 21.3 µm, followed by Aislyn and Cheche Lazaro both having a mean spine length of 20.8 µm. Vicky has a nearly half spine length of 11.7 µm, as compared to the those with long spine lengths. There was also a significant difference ($p<0.001$ α0.05 level) among the samples in terms of spine length. Observations mentioned appear to demonstrate evidence of variations among hybrids of this species specifically in terms of their pollen size and pollen spine length.

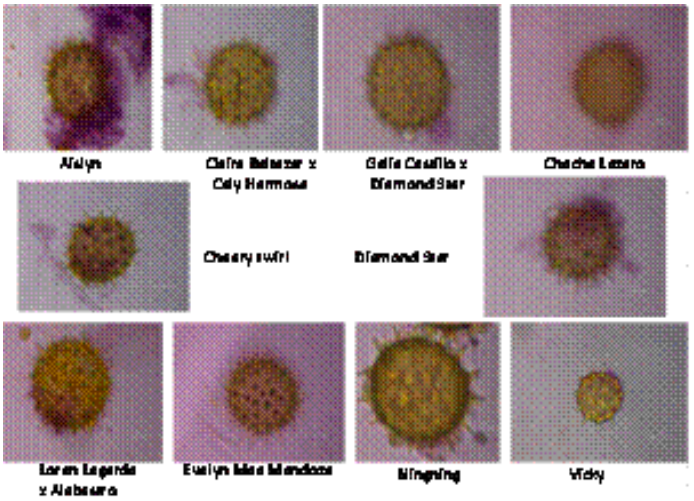

Figure 2. *Pollen from selected H. rosa-sinensishybrids(400x)*

Table 2. *Pollen size of the selected H. rosa-sinensis hybrids and their pollen size classes*

Hybrid	Ave Size (µm)	Pollen Size Class
Aislyn	112.9	very large
Cheche Lazaro	148.8	very large
Claire BaltazarxCely Hermosa	138.3	very large
Diamond Star	77.1	large
Gelia Castillo x Diamond Star	139.2	very large
Cheery twirl	118.8	very large
Ningning	135.0	very large
Evelyn Mae Mendoza	130.4	very large
Vicky	85.4	large
Loren Legarda x Estrella Alabastro	118.3	very large

The Hibiscus hybrids observed were noted to have spheroidal shape which is globose or ball-shaped (Fig. 3). Such shape description is in accordance with the study of Guerra et al. [2014].

Table 3. *Selected H.rosa-sinensis and their average spine length*

Hybrid	Ave Spine Length (µm)	Classification
Aislyn	20.8	long
Cheche Lazaro	20.8	long
Claire BaltazarxCely Hermosa	13.8	short
Diamond Star	14.2	short
Gelia Castillo x Diamond Star	18.8	long
Cheery twirl	21.3	long
Ningning	16.7	long
Evelyn Mae Mendoza	17.5	Long
Vicky	11.7	Short
Loren Legarda x Estrella Alabastro	21.3	Long

Minimal shape variation among the hybrids was noted based on Elliptic Fourier Coefficient Analysis using Shape software (Fig. 4). The effect of the quality of the photos of the samples upon processing using the software should not be discounted

as it could have affected the resulting outline of the shape after binarization. The pollen shapes follow similar contours although there are evident pleats and protrusions visible for several specimens. Some of these variations could be an indication of slight divergence to the overall shape of the pollen grains for this species. After numerous hybridizations of succeeding filial generations, these variations could be aggravated and possibly lead to eventual artificial selection leading to species of this species. It was also noted that all of the hybrids have pantoporate aperture type wherein pori occur globally on the pollen grain surface (Fig 5). In terms of sculpturing, they all exhibited echinate or spinelike type (Fig 6.). The same observations were reported by El-Kholy et al. [20] and Christensen [5]. These qualitative features still conform to the usual characters demonstrated by Hibiscus rosa-sinensis, hence such trait at the moment could not be an indication of phenotypic variation for this species unlike those examined in earlier sections. Perveen [11] mentioned that pollen polarity, symmetry, shape, type of aperture and sculpturing are important characters from a phylogenetic and evolutionary point of view. Specifically, he mentioned that the exine sculpturing is one of the constant features of a pollen grain by which genera or species may be distinguished from one another. In another study, pollen diameter, exine thickness, spine height and spine basal of 11 taxa of Hibiscus showed diversity and was used together with seed coat characters for their taxonomic evaluation [20]. It can then be said that if those characters vary among a certain taxon, such can be used to differentiate a group from the others. Table 4 summarizes the morphological characteristics of H. rosa-sinensis hybrids observed in this study.

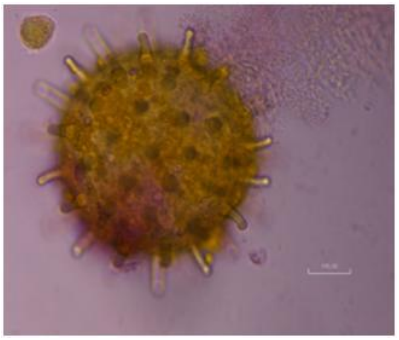

***Figure 3**. Spheroidal pollen shape of Vicky hybrid (400x)*

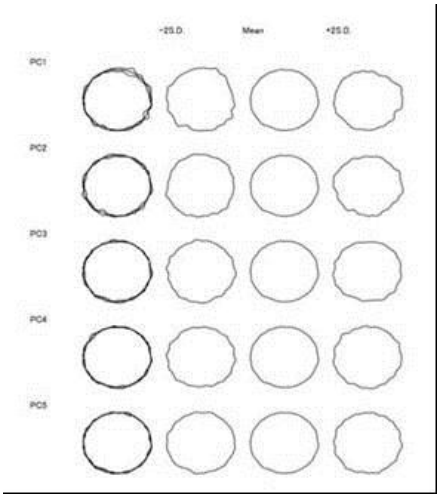

***Figure 4.** Pollen shape analysis based on based on Elliptic Fourier Coefficient Analysis using Shape software*

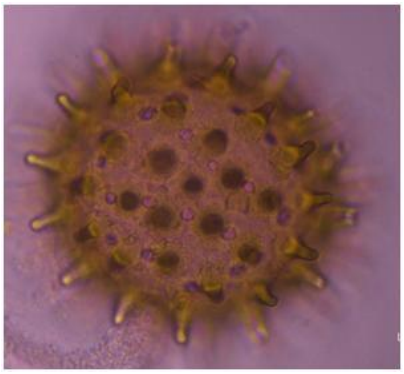

***Figure 5.** Pollen from Evelyn Mae Mendoza hybrid showing pantoporate aperture(400x)*

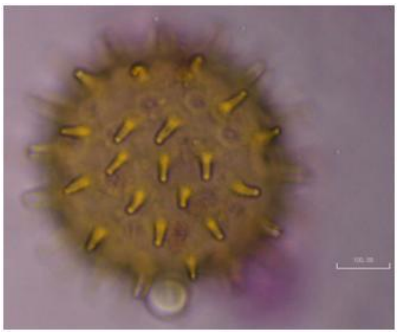

***Figure 6.**Echinate type of sculpturing of Gelia Castillo x Diamond Star hybrid (400x)*

***Table 4.**Summary table of morphological characteristics of the pollen of selected H. rosa-sinensis hybrids*

Hybrid	Aperture	Sculpturing	Pollen Shape	Pollen Size Class	Spine Length
Aislyn	pantoporate	echinate	spheroidal	very large	Long
Cheche Lazaro	pantoporate	echinate	spheroidal	very large	Ong
Claire Baltazar x Cely Hermosa	pantoporate	echinate	spheroidal	*very large	Short
Diamond Star	pantoporate	echinate	spheroidal	*large	short
Gelia Castillo x Diamond Star	pantoporate	echinate	spheroidal	very large	Long
Cheery twirl	pantoporate	echinate	spheroidal	very large	Long
Ningning	pantoporate	echinate	spheroidal	very large	Long
Evelyn Mae Mendoza	pantoporate	echinate	spheroidal	very large	Long
Vicky	pantoporate	echinate	spheroidal	*large	Short
Loren Legarda x Estrella Alabastro	pantoporate	echinate	spheroidal	very large	long

It can be noted that among the characters used, variation occurred in terms of pollen size and spine length. Among the hybrids, Claire Baltazar x Cely Hermosa somewhat differed based on the said characters, having very large size and short pollen spines. Furthermore, Diamond Star and Vicky hybrids have large pollen grain and short pollen spines. However, size and length of a certain structure in pollen, just like in any organism, can be a function of the ontogenetic development of the organism, thus, many consider them as not reliable parameters as mentioned above. Hence, in this study, pollen size and pollen spine length alone cannot be considered diagnostic characters for the selected H. rosa-sinensis hybrids. Conversely, Claire Baltazar x Cely Hermosa, Diamond Star and Vicky hybrids, cannot be considered a separate group per se. On the other hand, the observed qualitative diversity should not be disregarded and can be considered as evidences of variations. They are also probably another result of hybridization in progress just as the phenotypic variations in the flowers of the hybrids.

5. CONCLUSION

Palynology has been contributing to the systematics and phylogeny, especiallyof angiosperms. Some constant features of pollen, such as shape, sculpturing, and aperture provide important information (Perveen, 1998). Based on the data gathered, all pollen of the hybrids have pantoporate type of aperture, echinate type of sculpturing, and spheroidal shape. Pollen size ranged from large to very large and long to very long spine length. Among the characters, variation was observed in terms of pollen size and spine length of Claire Baltazar x Cely Hermosa, Diamond Star and Vicky hybrids. There was also significant difference among the samples in terms of these characters based on statistical analysis. However, these two characters cannot be considered diagnostic characters of the hybrids since such are features that are affected by ontogenetic development of the flower and laboratory treatments. It is noteworthy, however, that the variations observed may possibly be an on-going modification in pollen morphology as a result of hybridization just like in the phenotypic variations of the flowers from the hybrids used in this study. In the light of evolution, artificial selection, such as hybridization, could be an agent of microevolution and through time, may eventually lead to macroevolution specifically speciation. More intensive and detailed examination of other pollen features of H. rosa-sinesis hybrids including chemical analysis and use of larger sample size should be done if comprehensive characterization for distinguishing the pollen of the hybrids is to be realized.

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