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Morphological Characterization, Pollen Viability, and Germinability Evaluation of Two *Plumeria* Species in the University of the Philippines Los Baños

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The genus *Plumeria*, locally known as *kalachuci*, is a popular ornamental tree belonging to the Apocynaceae family. The University of the Philippines Los Baños (UPLB) maintains a collection of *Plumeria* trees scattered across its campus; however, the origin of these trees remains unclear due to a lack of proper documentation. This study conducted morphological characterization and pollen analysis to provide information for further research. Results revealed the presence of 2 *Plumeria* species in the campus. *P. obtusa* has a linear leaf shape with acute tips and revolute margins while *P. rubra* has a lanceolate leaf shape. Additionally, *P. obtusa* has a green peduncle while *P. rubra* has a peduncle ranging from red to dark red. In terms of floral traits, *P. obtusa* produces white flowers while *P. rubra* has flowers with a variety of colors including white, yellow, pink, and dark red. Pollen viability and pollen germinability analysis revealed that germinability varied significantly within and between species, whereas pollen viability remained similar for both. Both species exhibited high pollen viability observed through staining but low pollen germination. Furthermore, high pollen viability does not guarantee high germinability, as indicated by the weak correlation between these variables across species. To clarify the uncertain provenance of *P. rubra* variants, further research using molecular techniques and DNA markers for precise information is highly recommended.

Keywords: flower color, *kalachuchi*, morphological characterization, *Plumeria*, pollen viability, pollen germinability

Introduction

The genus *Plumeria* is distinguished by its thick, succulent branches and corky bark (Staples and Herbst 2005; Perez 2019). It belongs to the family Apocynaceae and is locally known as *kalachuchi*. The taxonomy of *Plumeria* is complex, as many species names used by collectors are incorrect or unverified (Criley 2009). Additionally, unconfirmed or mislabeled specimens in gardens contribute to the persistence of misnomers and uncertainty regarding the plants' origins (Perez 2019).

The University of the Philippines Los Baños (UPLB) maintains a collection of *Plumeria* trees scattered across its campus. *P. rubra* is native to Mexico and Central America, while *P. obtusa* is found across the islands of the Caribbean. Conducting morphological characterization and pollen analysis will generate information to stimulate further interest in these trees for research, landscaping, breeding, and educational purposes (Perez 2019). Morphological characterization is a cost-effective option for species identification and has proven useful in defining species relationships within specific genera and families (da Silva 2017).

In addition to evaluating the morphological traits of *Plumeria* trees in UPLB, this study focused on assessing pollen viability to support future breeding efforts. *P. rubra* has a chromosome number of 2n = 36, while *P. obtusa* has a chromosome number ranging from 2n =18 to 54 (Raghuvanshi and Chauhan 1971). Successful *Plumeria* hybridization

necessitates a consistent supply of viable pollen, which requires identifying parent plants with strong combining abilities and effective pollination traits. Evaluating pollen viability and germinability is crucial to enhance hybridization success (Perez and Criley 2013).

This study aimed to document and characterize the existing *Plumeria* species in UPLB, including evaluations of pollen viability and germinability. These assessments will support future hybridization programs and conservation efforts for this species. This preliminary study lays the foundation for future breeding programs, as characterizing flower traits and plant provenance provides valuable insights for developing targeted breeding strategies—for example, breeding *Plumeria* varieties with unique flower colors and forms, combined with desirable provenance traits for landscaping. Additionally, assessing *Plumeria* pollen quality will help determine its viability for hybridization, enhancing the effectiveness of breeding efforts.

Materials and Methods

Distribution of *Plumeria* in UPLB

The UPLB upper and lower campus hosts a total of 62 *Plumeria* trees, comprising 2 species: 36 *P. obtusa* trees and 26 *P. rubra* trees. The majority of *P. obtusa* trees, totaling 12, are located in Carabao Park. Conversely, a significant number of *P. rubra* trees, also 12, are situated in front of the Orchid Nursery Building along Andres P. Aglibut Avenue (Fig. 1). These are the 2 campus sites where the majority of the 2 species were planted. Additionally, the most prevalent variant of *P. rubra*, characterized by yellow flowers, includes 5 trees distributed across the campus.

Morphological Characterization

Multiple trees of *P. rubra* and *P. obtusa* are grown in different parts of the campus. Surveying and geotagging of individual *Plumeria* trees in the UPLB campus were conducted. This allowed for tagging and assigning unique numbers to individual trees, which would facilitate future location and sampling flowers and leaves. The *Plumeria* trees were geotagged and numbered from the upper campus site to the lower campus site.

For each plant, 50 flowers and leaves were randomly sampled with the aid of a man-lifter truck and articulated broom. Morphological characterization was performed to identify and assess the variation between the 2 species of *Plumeria*, with 42 individual trees successfully characterized. Morphological characterization was documented using a digital camera (DLSR, Canon EOS 6D), and morphometric analysis of digital photos of flowers and leaves was carried out using ImageJ software (version 1.54D).

Using the 6th edition of the Royal Horticultural Society (RHS) Colour chart (RHS 2015), different colors and shades of *Plumeria* flowers were assessed. Only the primary flower colors were evaluated.

A total of 15 qualitative and quantitative morphological descriptors were used to assess and evaluate the *Plumeria* trees in UPLB. These morphological traits include leaf length, leaf width, petiole length, leaf shape, leaf apex shape, leaf base shape, peduncle color, flower diameter, number of flower colors, petal shape, petal apex, intensity of flowering, petal arrangement, persistence of flowering, and RHS flower color group.

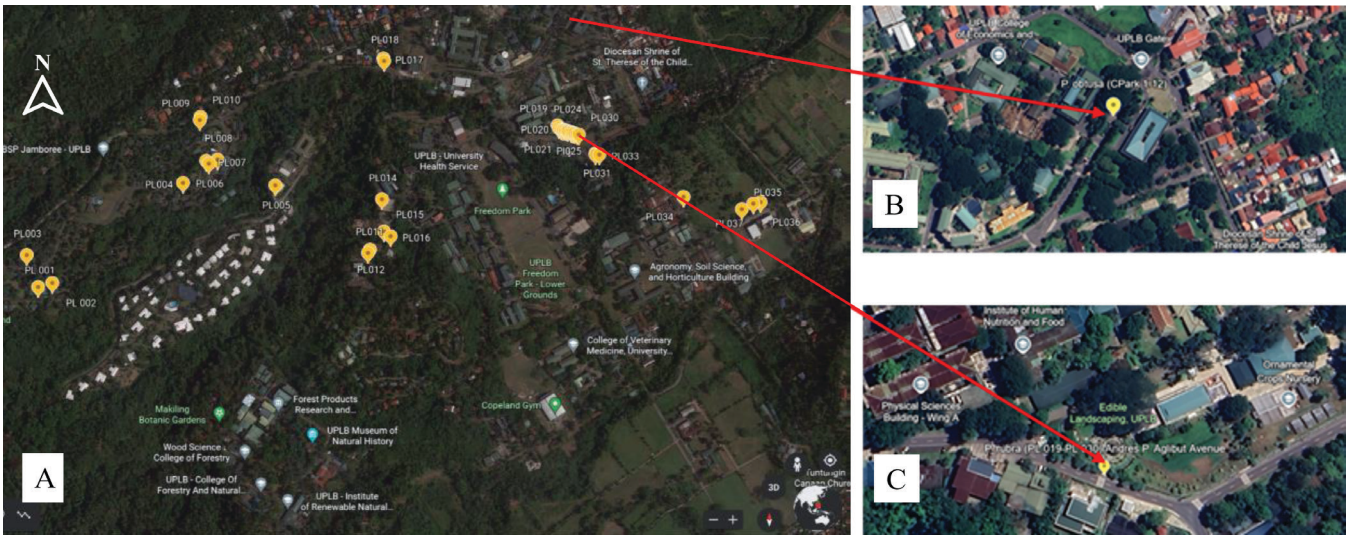

Fig. 1 Fig. 1 (A) Distribution of *Plumeria* in the University of the Philippines Los Baños with 2 prominent sites: (B) planted with *P. obtusa* at the Carabao Park and (C) *P. rubra* along Andres P. Aglibut Avenue, in front of the Orchid Nursery Building

Principal component analysis (PCA) was conducted to delineate the 2 species and determine the combination of traits that are unique to each species. Subsequently, cluster dendrograms were generated in R Statistical Software (R Core Team 2023, version 4.3.1 Beagle Scouts) using library packages. The graphs were generated and visualized using 'ggplot2' (Wickham 2016) and 'lattice' (Becker and Cleveland 1993) libraries. Additional library packages 'FactoMiner' (Husson et al. 2018) and 'factoextra' (Kassambara and Mundt 2017) were installed to perform the PCA and to generate individual factor maps and cluster dendrograms.

Pollen Viability and Germinability Evaluation

Sampling and analyses of pollen viability and germinability were conducted over the course of 3 yr (from 2021 to 2023). In the first year, evaluations were carried out on *P. rubra* flowers. The following year, both *P. obtusa* and *P. rubra* flowers were evaluated. In the third year, only *P. obtusa* flowers were assessed. Although the flower samples were collected in different years, all samples were collected during the month of April, when the peak of flowering occurs each year.

For pollen viability and germinability evaluations, each of the plants identified for this study was collected with pollen grains. Pollen was collected between 8:00 and 10:00 A.M. and only on sunny days. Pollen viability tests of different *Plumeria* genotypes were done using the Lugol's Iodine (I2KI) structural staining test, while pollen germinability evaluations were carried out on Brewbaker and Kwack (1964) medium. Pollen germinability evaluation was conducted using the modified procedures of Magdalita et al. (2016). The pollen germination medium was placed on depression slides.

Fifty pollen samples collected from 5 to 10 flowers were used per plant, replicated thrice. Pollen grains were observed under 100X magnification using a digital microscope (True Vision Microscopes, Inc., USA). Darkly stained pollen was interpreted as an indication of high pollen viability, while unstained and deformed pollen was indicative of infertile pollen (Wani et al. 2015). Fully stained, partially stained, and unstained pollen grains were counted for each of the 3 replications, and percent viability was computed using the formula:

$$\% \text{ viability} = (\text{number of fully/darkly stained pollen} / \text{total number of pollen samples}) \times 100$$

In vitro germination of pollen was carried out on Brewbaker and Kwack's medium (Brewbacker and Kwack 1964). This medium consisted of boric acid (1.6 mM), potassium nitrate (1.0 mM), calcium nitrate (1.3 mM), magnesium sulfate (0.8 mM), and sucrose (29 mM). Pollen grains from different *Plumeria* genotypes were germinated on this medium. Three drops of Brewbaker and Kwack's medium were dispensed in depression slides and were allowed to solidify for 5 min. Pollen grains were then brushed into the medium, and the slides were placed in plastic trays lined with moistened paper towels and covered to conserve moisture. The samples were left to germinate for 4 h.

Samples were viewed under a light compound microscope (True Vision Microscopes, Inc., USA), and the germinated pollen grains were visualized on a computer using ToupTek ToupView 3.7 software (ToupTek 2017). Pollen was considered germinated if the pollen tube was well-developed and elongated. Pollen tube length was measured and recorded 4 h after incubation, up to 8 h. Germinated and non-germinated pollen grains were counted, and percent germination was calculated for all treatments in the 3 replications using the formula:

$$\% \text{ germination} = (\text{germinated pollen} / \text{total number of pollen samples}) \times 100$$

Pollen viability and germination data were analyzed using the Mann-Whitney U test to determine differences across *P. rubra* and *P. obtusa* species. The correlation between pollen viability and germination for *P. obtusa* and *P. rubra* was determined using the Spearman's rho correlation.

Results and Discussion

Based on the characterization, a striking difference between the two species was observed in the leaf characteristics. *P. obtusa* has an obovate leaf shape with a non-pointed leaf apex, while *P. rubra* has a lanceolate leaf shape (Fig. 2). Another distinct characteristic between the 2 *Plumeria* species is the peduncle color. *P. obtusa* has a green peduncle, while the *P. rubra* peduncle ranges from reddish green to red and dark red (Fig. 3).

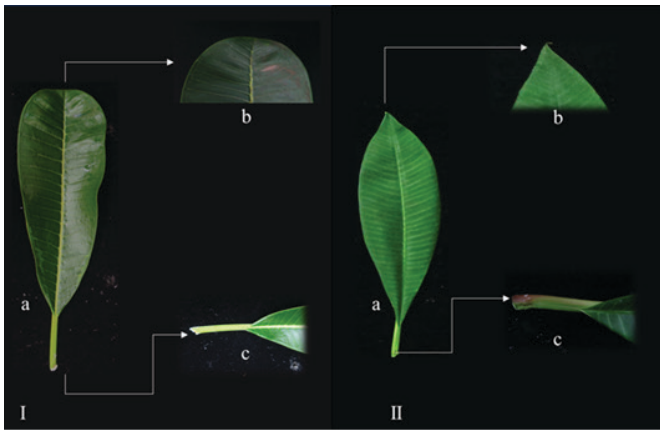

Fig. 2 Leaf qualitative morphological characteristics of (I) *Plumeria obtusa* and (II) *Plumeria rubra* found in the University of the Philippines Los Baños; (a) leaf shape and glossiness, (b) leaf apical shape, (c) petiole color

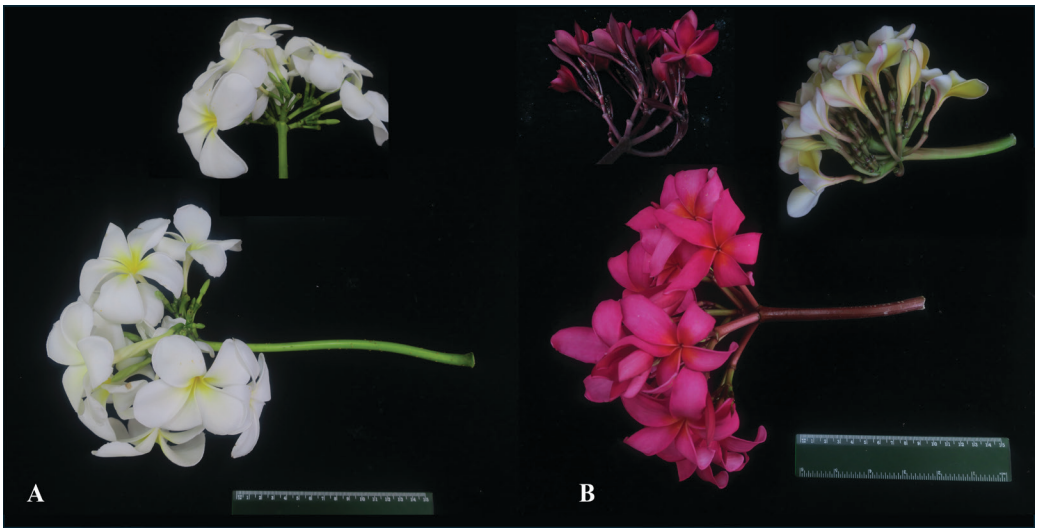

Fig. 3 Peduncle characteristics of (A) *Plumeria obtusa* and (B) *Plumeria rubra* found in the University of the Philippines Los Baños

In terms of flower characteristics, *P. obtusa* has white-colored flowers, while *P. rubra* has flowers with varying colors, ranging from white, yellow, and pink to dark red. In addition, *P. rubra* petals are lanceolate with a closed and tight arrangement, whereas the *P. obtusa* petals are obovate with a loose arrangement (Fig. 4).

Over a 3-yr period, it was observed that *P. rubra* flowers early between February and March, with flowers emerging from leafless trees (Fig. 5). The appearance of leaves occurs between March and April, coinciding with the commencement of flowering of *P. obtusa*.

Unlike most variants of *P. rubra*, *P. obtusa* retains most of its leaves throughout the year and only sheds most of its flowers in November to January, while *P. rubra* undergoes complete leaf defoliation in the same period. Murashige (1966) also documented the deciduous behavior of *Plumeria* during a similar time of the year.

Fig. 4 Flower characteristics of (A) *Plumeria obtusa* and (B) *Plumeria rubra* variants found in the University of the Philippines Los Baños

Fig. 5 Leaf defoliation on *Plumeria rubra* trees found in the University of the Philippines Los Baños; (a) no flowers and very few leaves, (b) no flowers but plenty of leaves, and (c) no flowers and leaves

On the other hand, partial shedding of the leaves and flowers on *P. obtusa* was observed to coincide with the presence of sooty molds and infestation by scale insects. This was observed in 2 groups of *P. obtusa* in Carabao Park and the Student Union Building. The occurrence of sooty molds and scale insects on *P. obtusa* trees greatly reduced the flowers and partially caused leaf defoliation. The presence of scale insects and sooty mold was not observed in *P. rubra* trees.

In terms of flower colors assessed using the RHS Colour Chart, results show a total of 8 different color shades among *P. rubra* (Fig. 6).

In terms of quantitative characters of leaves and flowers, *P. obtusa* has shorter but wider leaves with a longer petiole length and larger flowers compared to *P. rubra*. *P. obtusa* has an average leaf length of 25.87 cm, while *P. rubra* has longer leaves, averaging 29.01 cm. *P. obtusa* has an average leaf width of 14.29 cm, whereas *P. rubra* has an average leaf width of only 7.77 cm. *P. obtusa* has an average petiole length of 4.68 cm, while *P. rubra* has an average of 3.92 cm. The flower diameter of *P. obtusa* is larger than that of *P. rubra* (Table 1).

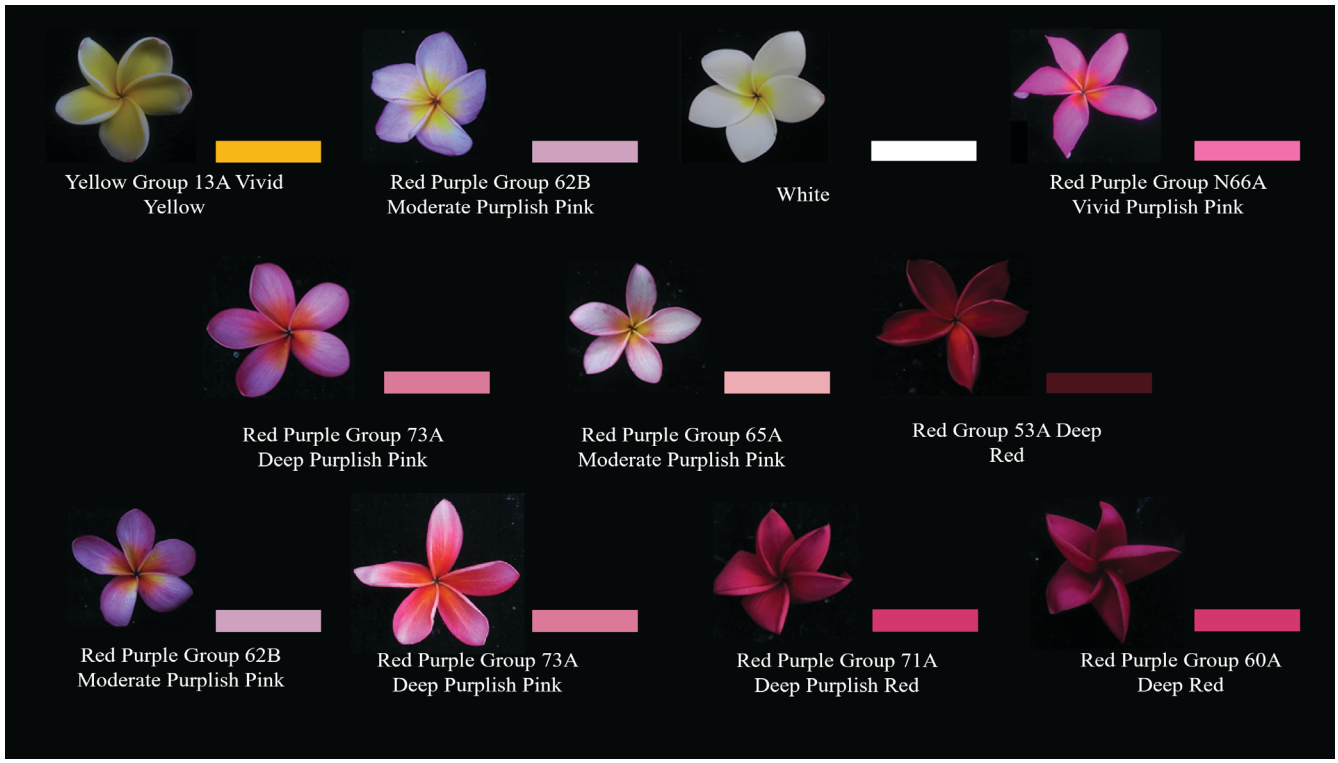

Fig. 6 Color group classification of *P. rubra* variants found in the University of the Philippines Los Baños, according to the Royal Horticultural Society Colour Chart, 6th edition (RHS 2015)

Table 1 Leaf and flower quantitative characters of *Plumeria* species found in the University of the Philippines Los Baños

Plumeria species	Leaf length (cm)	Leaf width at the widest part (cm)	Petiole length (cm)	Flower diameter (cm)
P. obtusa	25.87 ± 0.77	14.29 ± 0.36	4.68 ± 0.16	7.42 ± 0.16
P. rubra	29.01 ± 1.12	7.77 ± 0.20	3.92 ± 0.14	5.95 ± 0.92

Principal Component Analysis

Out of 62 total geotagged *Plumeria* trees, 42 were successfully characterized. The remaining trees could not be characterized due to logistical challenges requiring specialized equipment such as a man-lifter truck and an articulated broom to sample flowers at height. Additionally, some *Plumeria* trees were not flowering at the time of sampling due to obstructions from climbing weedy vines and significant loss of branches due to strong winds. Due to the observed high degree of similarity among *P. obtusa* trees, the sample size was reduced, prioritizing those for pollen evaluation. Results from the individual factor map show the closeness and relatedness of individual *Plumeria* trees to each other. The variations in flower color within the species also contributed to the relative distance of individual trees in the individual factor map. The map revealed 2 groups: the first group (on the left) comprised 24 *P. obtusa* individuals that are close and overlapping with each other, while the second group (on the right) comprised *P. rubra* individuals. Although the *P. rubra* individuals are grouped together, they are relatively distant from each other. The relative distance within the *P. rubra* variants could be attributed to variations in flower number, flower color, and petal shape. The close grouping of *P. obtusa* individuals reflects a more uniform set of qualitative and quantitative characteristics, such as leaf and flower size, and white-colored flowers for the species. In contrast, the broader spread among *P. rubra* individuals highlights the degree of diversity and variants, mainly due to floral characteristics having different sizes, shapes, and colors (Fig. 7). As a result, the cluster dendrogram revealed 2 major clusters: *P. obtusa* and *P. rubra* (Fig. 8). The first cluster on the left (*P. obtusa*) is composed of 24 *Plumeria* trees, while the second cluster on the right (*P. rubra*) is composed of 18 *Plumeria* trees that were successfully characterized.

Pollen Viability and Germinability Evaluation

In terms of pollen viability and germinability, the first year of the study focused solely on *P. rubra*. The results revealed that pollen viability varied significantly among the 10 *P. rubra* variants examined in May 2021, with a relatively high percent viability ranging from 82.96% (PL 019) to 94.92% (PL 021). However, overall pollen germinability was relatively low and ranged only from 16.14% to 30.78% (Table 2). The results

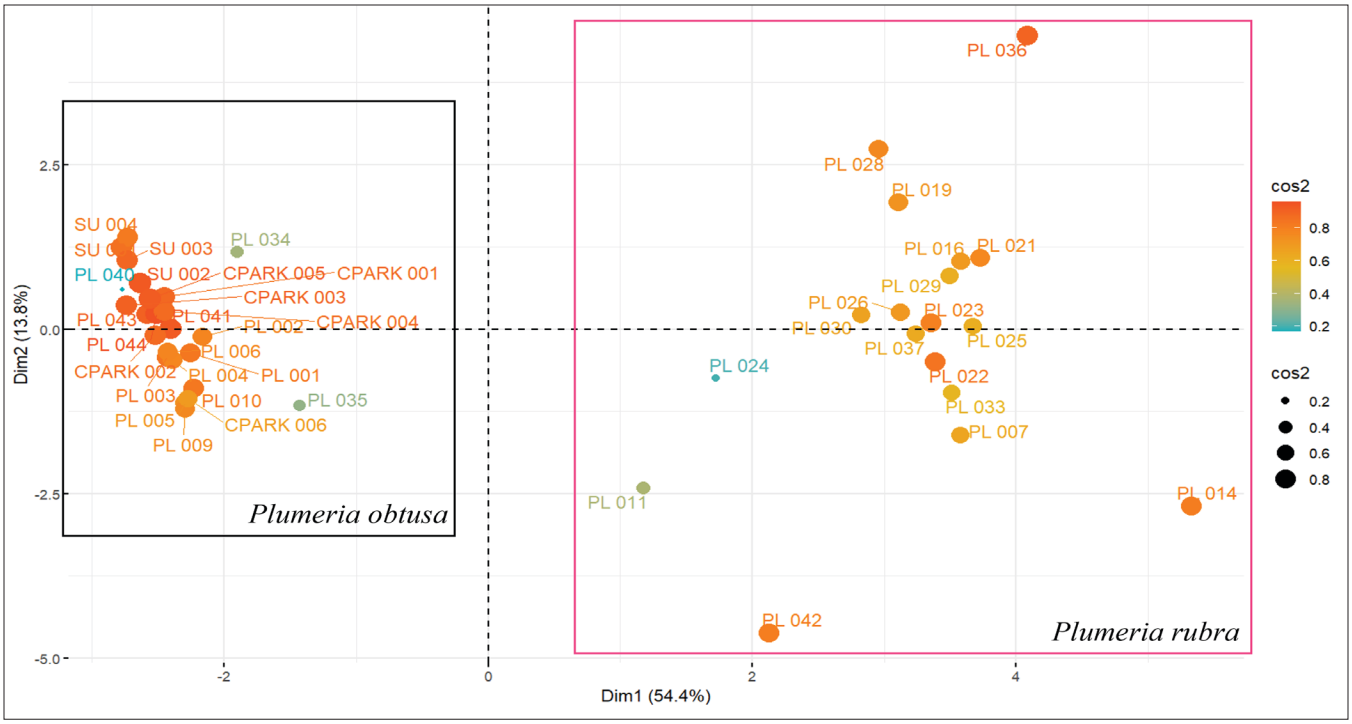

Fig. 7 Individual factor map of *Plumeria obtusa* and *Plumeria rubra* found in the University of the Philippines Los Baños. The size (0.2 to 0.8) and color gradient (blue to red orange) of the points (cos²) indicate the strength of their contributions to Dim1 and Dim2, with larger and darker-colored points being better represented in the factor map.

suggest that pollen viability does not correlate directly with pollen germinability. Spearman correlation analysis revealed a very weak correlation between germination and pollen viability for both *P. obtusa* (r = 0.117) and *P. rubra* (r = 0.154) (Table 3).

Generally, pollen viability indicated by staining and pollen germination have been shown to have a direct linear relationship. However, this varies across species (Sulusoglu and Cavusoglu 2014). Pollen staining tests rely on the staining of pollen components to rapidly estimate potential viability. For I2KI tests, the intensity of staining is related to the amount of starch in a pollen grain. Thus, intensely stained pollen grains are thought to be viable, whereas poorly stained pollen grains are thought to be less viable or inviable due to a lack of internal starch components. On the other hand, in vitro pollen germination tests are considered a more direct measure of fertility as viability is assessed based on pollen tube growth. In this study, it was observed that pollen staining with I2KI is a poor indicator of pollen viability.

Pollen tube length is crucial as it affects the ability to grow long enough to reach and fertilize an ovule, but this factor was not measured in this study. Notably, *P. rubra* trees

Table 2 Percent pollen viability and germination of *Plumeria rubra* collected in May 2021 at the Orchid Nursery Building, Andres P. Aglibut Avenue, University of the Philippines Los Baños

Plumeria tree number	*Plumeria* species	Percent viability (%)	Percent germination (%)
PL 019	*P. rubra*	**82.96 ± 5.80 b**	21.41 ± 6.84 b
PL 020	*P. rubra*	92.13 ± 4.10 ab	22.84 ± 7.73 b
PL 021	*P. rubra*	**94.92 ± 3.60 a**	25.22 ± 7.69 b
PL 022	*P. rubra*	94.52 ± 1.10 a	27.29 ± 8.90 ab
PL 023	*P. rubra*	93.45 ± 1.50 a	18.75 ± 8.22 bc
PL 024	*P. rubra*	87.75 ± 4.80 ab	16.14 ± 0.95 c
PL 025	*P. rubra*	89.78 ± 1.30 ab	27.40 ± 4.41 ab
PL 026	*P. rubra*	89.80 ± 5.30 ab	20.76 ± 4.95 bc
PL 029	*P. rubra*	94.07 ± 2.30 a	30.78 ± 11.44 ab
PL 030	*P. rubra*	91.77 ± 1.40 b	21.03 ±1.21 bc

Values with the same letters are not significantly different using LSD Pairwise comparison test at $P < 0.05$. Values in bold face are the highest and lowest values observed per parameter.

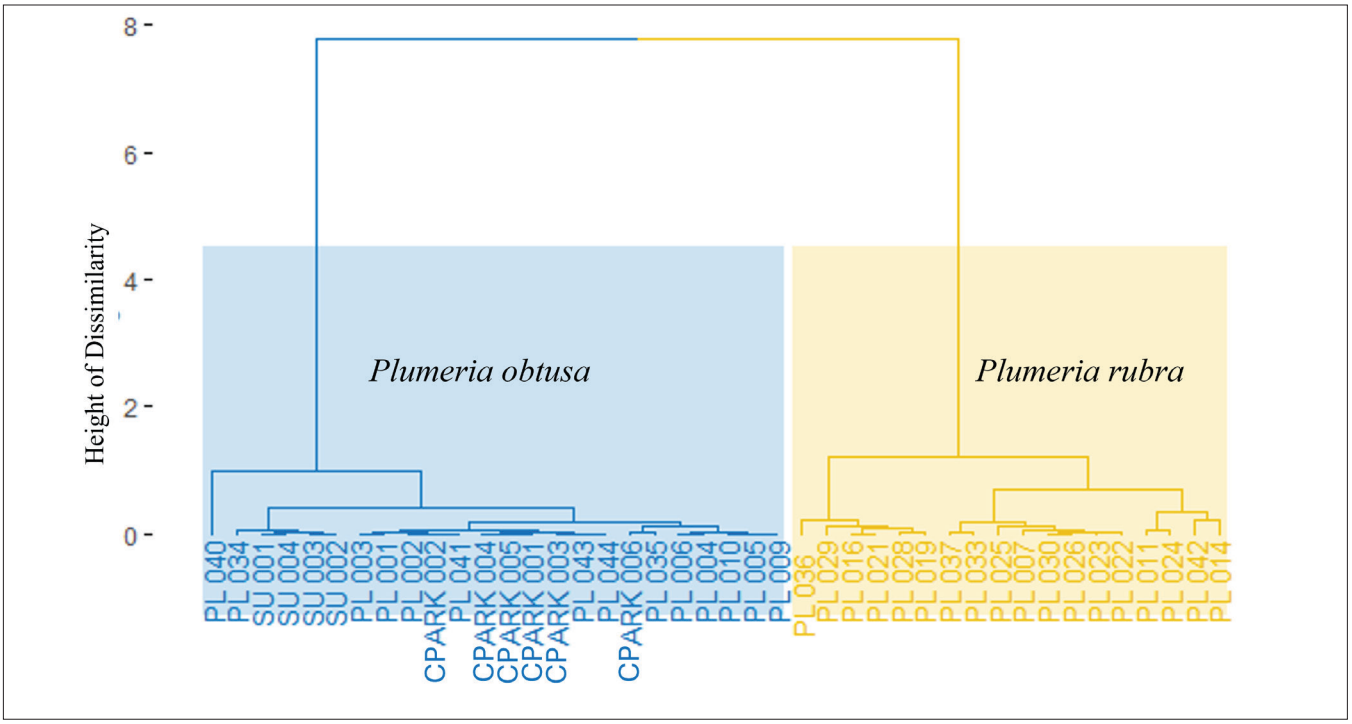

Fig. 8 Cluster dendrogram of individual Plumeria trees found in the University of the Philippines Los Baños and successfully characterized. Two major groupings composed of 2 species are shown: *P. obtusa* with 24 individuals, and *P. rubra* with 18 individuals.

Table 3 Spearman's rho correlation coefficients for pollen viability and germination of *Plumeria obtusa* and *Plumeria rubra* found in the University of the Philippines Los Baños

P. obtusa	Viability	*P. rubra*	Viability
Germination	0.117 (very weak correlation)	Germination	0.154 (very weak correlation)

such as PL 019 exhibited significantly low pollen viability and lower pollen germinability. In contrast, PL 22 demonstrated high pollen viability (94.52%) but low pollen germinability (18.75%). In the second year of the study, pollen samples were collected from both *P. obtusa* and *P. rubra*. Results for this year indicated that regardless of species and variants within the species, pollen viability still varied (Table 4). In the third year of the study, all flower samples were obtained from *P. obtusa* trees. The results on pollen viability showed a high degree of variability, ranging from 76.78% to 92.44% (Table 5). One peculiarity of this year was the increase in percent germination compared to previous years.

Pollen germination in *P. obtusa* was significantly different from that of *P. rubra* (Table 6). However, pollen viability by staining was similar across both *Plumeria* species. The results of this study corroborate with the findings of Perez and Criley (2013), who also observed variation in pollen germination across different *Plumeria* species.

Table 4 Percent pollen viability and germination of *Plumeria obtusa* and *Plumeria rubra* collected in May 2022 in various sites within the University of the Philippines Los Baños

Plumeria tree number	*Plumeria* species	Percent viability (%)	Percent germination (%)
PL 001	*P. obtusa*	73.64 ± 0.69 ab	43.67 ± 2.30 a
PL 002	*P. obtusa*	70.94 ± 1.94 ab	42.93 ± 1.99 a
PL 003	*P. obtusa*	86.82 ± 0.24 ab	54.76 ± 0.73 a
PL 007	*P. rubra*	90.06 ± 0.47 ab	25.95 ± 1.13 b
PL 016	*P. rubra*	84.31 ± 0.14 ab	21.55 ± 1.11 bc
PL 034	*P. obtusa*	87.92 ± 0.28 ab	49.64 ± 0.85 a
PL 036	*P. rubra*	87.78 ± 0.79 ab	15.19 ± 0.24 c
PL 036b	*P. rubra*	**91.12 ± 0.26 a**	37.22 ± 1.71 ab
PL 037	*P. obtusa*	89.32 ± 0.45 ab	**12.19 ± 0.97 c**
PL 040	*P. obtusa*	87.91 ± 0.59 ab	25.91 ± 0.87 b
PL 043	*P. obtusa*	82.66 ± 0.81 ab	29.04 ± 1.00 b
PL 044	*P. obtusa*	**69.00 ± 0.43 b**	25.30 ± 0.63 b

Values with the same letters are not significantly different using LSD Pairwise comparison test at $P < 0.05$. Values in bold face are the highest and lowest values observed per parameter.

Table 5 Percent pollen viability and germination of *Plumeria obtusa* collected in July 2023 at Carabao Park (CPark) and the vicinity of the Student Union Building (SU), University of the Philippines Los Baños

Plumeria tree number	*Plumeria* species	Percent viability (%)	Percent germination (%)
CPark 001	*P. obtusa*	87.81 ± 0.57 a	50.65 ± 5.70 ab
CPark 002	*P. obtusa*	82.30 ± 0.30 b	48.23 ± 2.70 ab
CPark 003	*P. obtusa*	91.32 ± 0.47 a	40.80 ± 3.74 b
CPark 004	*P. obtusa*	89.10 ± 0.29 a	52.23 ± 7.18 ab
CPark 005	*P. obtusa*	91.90 ± 0.38 a	58.51 ± 8.70 ab
CPark 006	*P. obtusa*	89.54 ± 0.47 a	31.32 ± 0.70 c
CPark 007	*P. obtusa*	76.78 ± 0.70 c	30.97 ± 1.55 c
CPark 008	*P. obtusa*	92.99 ± 1.84 a	34.26 ± 2.15 bc
CPark 009	*P. obtusa*	91.85 ± 0.40 a	41.06 ± 6.30 bc
CPark 010	*P. obtusa*	82.67 ± 1.16 b	33.11 ± 2.13 bc
CPark 011	*P. obtusa*	91.99 ± 0.22 a	46.06 ± 4.21 b
CPark 012	*P. obtusa*	87.48 ± 0.65 a	50.13 ± 2.79 ab
SU 001	*P. obtusa*	92.32 ± 0.72 a	54.49 ± 4.70 ab
SU 002	*P. obtusa*	89.16 ± 2.50 a	69.17 ± 3.55 a
SU 003	*P. obtusa*	90.23 ± 1.39 a	58.34 ± 6.71 ab
SU 004	*P. obtusa*	92.42 ± 1.62 a	53.01 ± 5.83 ab

Values with the same letters are not significantly different using LSD Pairwise comparison test at $P < 0.05$.

Table 6 Pollen viability by staining and germination of *Plumeria obtusa* and *Plumeria rubra* found in the University of the Philippines Los Baños

Plumeria species	Pollen viability by staining (%)	Pollen germination (%)
P. obtusa	86.17 ± 7.00 a	43.16 ± 13.22 a
P. rubra	90.36 ± 3.64 a	23.68 ± 5.87 b
p-value	0.054	0

Values with the same letters are not significantly different using LSD Pairwise comparison test at $P < 0.05$.

Conclusion

Plumeria in UPLB consists of 2 species: *P. obtusa* and *P. rubra*. Key distinguishing characteristics between the 2 species are leaf shape and glossiness, flower color, peduncle color, and the timing of flowering initiation and leaf defoliation. *P. obtusa* has larger leaves and flowers compared to *P. rubra*. However, while *P. obtusa* only has white-colored flowers, *P. rubra* has multiple variants with different flower colors. Pollen germinability differs significantly within and between the species, while pollen viability is similar for both species. Generally, high pollen viability indicated by staining and low germination were observed in both species. Furthermore, high pollen viability does not necessarily ensure high germinability, as shown by the very weak correlation between these variables. The identification of *P. rubra* variants remains challenging due to uncertain provenance, suggesting the need for molecular techniques and DNA markers such as DNA barcoding for accurate identification.

Acknowledgments

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