

MORPHOLOGICAL VARIATION AND ECOLOGICAL ADAPTATION IN *PLUMERIA PUDICA*

Kavan Shukla, Kunal N. Odedra* and B. A. Jadeja

Department of Botany, M.D. Science College, Porbandar, Gujarat 360575, India

*Email: kunal.n.odedra1@gmail.com

Received: 23/10/2025, Revised: 24/02/2026, Approved: 02/03/2026

ABSTRACT

The present study aimed to conduct a comprehensive integrative morphological and micromorphological assessment of *Plumeria pudica* Jacq., a widely cultivated ornamental shrub of the family Apocynaceae. Specifically, the research sought to evaluate morphological diversity among seventeen accessions collected from diverse agroclimatic zones of Gujarat and Daman, India, analyze quantitative vegetative and reproductive traits along with qualitative characters, and investigate micromorphological features to understand trait variation, integration, and adaptive significance. Seventeen accessions were systematically examined for quantitative traits including plant height, leaf dimensions, branching pattern, and floral parameters, along with qualitative morphological characteristics. Scanning electron microscopy (SEM) was employed to analyze micromorphological structures of pollen, petal, leaf, stem, and root tissues. Statistical analyses, including Simpson's and Shannon–Wiener diversity indices, correlation analysis, and hierarchical clustering, were performed to assess intraspecific variation, diversity patterns, and coordinated trait development. Substantial intraspecific variation was observed, particularly in plant height (122–579 cm), leaf size, branching architecture, and floral traits, supported by high diversity index values. SEM analysis revealed distinct pollen exine ornamentation, specialized petal epidermal architecture, variations in stomatal distribution, and root mycorrhizal associations indicative of adaptive strategies. Strong correlations and modular clustering demonstrated coordinated development between vegetative and floral traits. The findings highlight significant phenotypic plasticity and ecological adaptability in *Plumeria pudica*, providing baseline information for conservation, breeding, and horticultural improvement efforts.

Keywords: Floral micromorphology; Morphological diversity; *Plumeria pudica*; Scanning electron microscopy; Stomatal ecology.

INTRODUCTION

Plumeria pudica Jacq., a perennial evergreen shrub of the family Apocynaceae, is highly valued as an ornamental plant for its abundant flowering, attractive foliage, and adaptability to tropical and subtropical environments. Native to tropical America, it is now widely cultivated worldwide, especially in gardens, landscapes, and urban green spaces (Kobayashi *et al.*, 2019). While the genus *Plumeria* is well known for its striking floral forms and horticultural versatility, *P. pudica* has received comparatively limited attention in integrative morphological and micromorphological studies despite its ecological and ornamental importance.

Investigations of plant form and structure are crucial for understanding diversity, taxonomy, and adaptive strategies. Morphometric analyses of traits such as plant height, branching, leaf dimensions, and floral architecture can reveal patterns of phenotypic plasticity and intraspecific variation (Barthlott *et al.*, 2018). At a finer scale, micromorphological approaches, particularly Scanning Electron Microscopy (SEM), provide insights into features such as stomatal type, petal epidermis, and pollen sculpturing, which are taxonomically informative and functionally linked to pollination and ecological adaptation (Prabhakar, 2004). However, existing studies on *P. pudica* largely emphasise its ornamental value and descriptive traits (Rao & Rajput, 2012), without systematically addressing intra-population variability or the

adaptive role of structural features. This gap restricts our understanding of how vegetative and reproductive traits interact, how micromorphological characteristics influence ecological performance, and how these traits may inform conservation and breeding strategies.

In this context, it is hypothesized that the morphological and micromorphological traits of *P. pudica* vary significantly among accessions due to genotype–environment interactions, reflecting both phenotypic plasticity and adaptive specialization. To test this, the present study assesses phenotypic variation across populations, explores correlations and trade-offs between vegetative and reproductive traits, and characterizes micromorphological features of pollen, petals, stems, roots, and stomata using SEM. Furthermore, it interprets the ecological and adaptive significance of these traits in relation to growth, reproduction, and environmental resilience. By integrating quantitative morphometric analyses with micromorphological characterization, this study provides a comprehensive account of structural variability in *P. pudica*, thereby contributing to Apocynaceae systematics while offering practical insights for horticultural improvement and conservation of this widely cultivated species.

METHOD

2.1. Plant Material and Study Sites

Seventeen accessions of *P. pudica* were collected from diverse locations across Gujarat and the Union Territory of Daman, India, based on observable morphological variations (Table 1). The accessions were coded PP1–PP17, and their GPS coordinates were recorded. The collected plants were maintained under uniform garden conditions for further characterization of morphological, cytological, biochemical, and molecular traits.

Table 1. Collection sites of *P. pudica* accessions across Gujarat and Daman

No.	Accession no.	Location	Latitude	Longitude
1.	PP1	Punit Van, Gandhinagar	23.237° N	72.647° E
2.	PP2	Sector 2/D, Gandhinagar	23.198° N	72.648° E
3.	PP3	Indroda Park, Gandhinagar	23.193° N	72.653° E
4.	PP4	Sector 21, Gandhinagar	23.232° N	72.663° E
5.	PP5	Sector 14, Gandhinagar	23.237° N	72.634° E
6.	PP6	Randheja	23.292° N	72.641° E
7.	PP7	Pethapur	23.265° N	72.672° E
8.	PP8	Amarnath Society, Kadi	23.290° N	72.342° E
9.	PP9	Kalol	23.241° N	72.493° E
10.	PP10	Amrapali Society, Vadodara	22.276° N	73.184° E
11.	PP11	Dharmapur, Valsad	20.54° N	73.179° E
12.	PP12	Daman	20.409° N	72.83° E
13.	PP13	Vijapur, Mehsana	23.56° N	72.75° E
14.	PP14	Veer residency, Mahudi	21.092° N	71.77° E
15.	PP15	Lodra village, Mansa	23.462° N	72.718° E
16.	PP16	Porbandar	21.641° N	69.629° E
17.	PP17	BKNMU, Junagadh	21.515° N	70.456° E

The collection sites fall under different agroclimatic zones of Gujarat and Daman. Gandhinagar, –Kalol, Vijapur, and Pethapur (North Gujarat Zone, GJ-4) experience an arid to semi-arid climate with 625–875 mm annual rainfall and sandy loam to loamy soils. Vadodara (Middle Gujarat Zone, GJ-3) has a semi-arid climate, ~939.6 mm rainfall, and alluvial sandy loam soils. Valsad and Daman (South Gujarat Heavy Rainfall Zone, GJ-1) are characterized by a humid coastal climate with 1,800–2,100 mm rainfall and coastal alluvial soils. Junagadh and Porbandar (South Saurashtra Zone, GJ-7) experience a dry sub-humid climate, with 800–1,000 mm rainfall and medium black soils.

2.2. Quantitative Morphological Characters

A total of 17 quantitative traits were recorded for each accession. Vegetative traits included: (1) plant height, (2) canopy spread (north–south), (3) canopy spread (east–west), (4) number of branches, (5) number of branchlets, (6) number of leaves, (7) leaf length, (8) leaf width, (9) petiole length, and (10) leaf thickness. Reproductive traits included: (11) number of inflorescences, (12) number of flowers per inflorescence, (13) flower length, (14) flower pedicel length, (15) petal length, (16) petal width, and (17) floral eye zone diameter. Standard procedures were followed: plant height was measured using a meter scale; branching and leaf number were manually counted; leaf dimensions were calculated from five replicate leaves per plant; and floral traits were measured at full bloom. All measurements were expressed as mean values in centimeters or counts, depending on the parameter.

2.3. Diversity Indices

2.3.1. Quantitative Morphological Traits and Diversity Indices

A total of 17 morphological traits were quantified across all *Plumeria pudica* accessions. These comprised vegetative traits such as plant height, canopy spread (north–south and east–west), branch number, branchlet number, and leaf dimensions, as well as floral traits including flower length, petal size, inflorescence count, pedicel length, and floral eye zone diameter. Standard procedures involving meter scales, manual counting, and replicated measurements were employed to ensure accuracy. Vegetative traits (e.g., plant height, width, branch, and leaf counts) were organized into frequency classes, while continuous variables such as leaf size, petiole length, flower dimensions, and petal characteristics were grouped into quantitative ranges. To assess intra-specific morphological variation, diversity indices were computed for each trait independently following Magurran, (2013).

Simpson's Diversity Index (D): This index estimates the probability that two randomly selected individuals from a sample belong to the same category (Simpson, 1949). It was calculated as:

$$D = \sum \frac{n_i(n_i - 1)}{N(N - 1)}$$

Description:

n_i : the number of..... etc downwards

N : is the total number of individuals

D : is the range from 0 to 1, with lower values indicating higher diversity

Shannon–Wiener Diversity Index (H'): This index measures both richness and evenness of trait categories (Shannon & Weaver, 1949). It was calculated using:

$$H' = - \sum (p_i \ln p_i)$$

Description:

p_i : the proportion of individuals in the i -th category

S : the total number of categories

Values of $H' > 3.5$ indicate high diversity, 1.5–3.5 reflect moderate diversity, and < 1.5 indicate low diversity.

Pielou's Evenness Index (J'): This index evaluates the uniformity of distribution across categories (Pielou, 1966). It was calculated as:

$$J' = \frac{H'}{\ln(S)}$$

Description:

H' : the Shannon–Wiener index

S: total number of categories

Values approaching 1 indicate perfect evenness, while values near 0 suggest dominance by one or a few categories.

2.3.2. Detection of Variation among Quantitative Characters

To investigate interrelationships among the quantitative traits of *P. pudica*, a correlation-based hierarchical clustering approach was employed. This method enabled the identification of trait groupings based on pairwise correlations, providing insights into potential functional and developmental linkages. This method quantifies the strength and direction of linear relationships between continuous variables, with correlation coefficients (r) ranging from -1 to +1. A value of +1 indicates a perfect positive linear relationship, -1 represents a perfect negative linear relationship, and 0 denotes the absence of linear association. Correlation matrices were generated using OriginPro software, with significance levels set at $p < 0.05$. The resulting matrices provided the foundational similarity measures for subsequent multivariate analyses, including cluster analysis and principal component analysis (Fang, 2024).

2.3.3. Data Preparation and Correlation Matrix Computation

Quantitative data for 17 traits were compiled across all accessions. These included plant length (PL), number of branches (NB), number of branchlets (NBL), number of leaves (NL), average leaf length (ALL), average leaf width (ALW), petiole length (LP), average leaf thickness (ALT), number of inflorescences per plant (NI), number of flowers per plant (NFP), flower length (LF), flower pedicel length (LFP), floral eye zone diameter (FED), petal length (LP2), and petal width (WP). Pairwise relationships among traits were quantified using Pearson's correlation coefficients, which measure the strength and direction of linear associations, ranging from -1 (perfect negative correlation) to +1 (perfect positive correlation). The resulting correlation matrix formed the basis for subsequent clustering analyses (Fang, 2024). Because hierarchical clustering requires a distance matrix, the correlation matrix was transformed using the formula:

$$Distance_{ij} = 1 - |Correlation_{ij}|$$

This approach ensured that traits with high positive or negative correlations were considered closer in the clustering process. The use of absolute correlation values enabled recognition of both synergistic and trade-off relationships, as both are functionally informative (RR, 1958).

2.4. Qualitative Morphological Characters

Twenty-two qualitative traits were observed across accessions to capture variability in growth habit, stem texture, leaf morphology, floral features, and venation patterns (Table 2). Traits such as leaf shape, apex, and base were classified using botanical descriptors, while color-based traits (leaf, flower, eye zone, receptacle) were recorded under uniform daylight conditions. Floral aestivation, venation, and inflorescence type were also documented.

Table 2. Qualitative morphological traits studied in *P. pudica* (Beentje, 2010)

No.	Trait	Description
1.	Type of plant	From herbs, shrubs, trees, climbers, and creepers
2.	Plant growth habit	From round, spreading, pyramidal, oval, conical, vase, columnar, open, weeping, and irregular types
3.	Stem texture	From herbaceous(soft) and woody(hard)
4.	Stem anthocyanin	From pelargonidin, cyanidin, peonidin, delphinidin,

coloration	malvidin, and petunidin
5. Leaf color	According to visual observations
6. Leaf growth on the stem	Acropetal or basipetal
7. Texture of the leaf	From smooth, tough, sticky, rough, mealy, and waxy
8. Leaf shape	From linear, ovate, elliptical, cordate, lanceolate, acicular, reniform, orbicular, lanceolate, hastate, lyrate, spatulate, cuneate.
9. Type of stomata	From anomocytic, paracytic, anisocytic, and diacytic.
10. Leaf base shape	From attenuate, auriculate, clasping, cordate, cuneate, hastate, oblique, peltate, perfoliate, rounded, and sagittate.
11. Leaf apex shape	From acuminate, acute, aristate, caudate, cuspidate, emarginate, mucronate, obtuse, and retuse.
12. Type of inflorescence	From raceme, corymb, spike, catkin, spadix, simple umbel, compound umbel, capitulum, hypanthodium, uniparous cyme, biparous cyme, multiparous cyme.
13. Petal arrangement of flowers	From valvate, twisted, imbricate, quincuncial, and vexillary.
14. The color of the flower	According to visual observations
15. Color of the flower's eye zone	According to visual observations
16. Color of the receptacle	According to visual observations
17. Shape of petals	From oval, obovate, cup-shaped, trumpet-shaped, intricate, ruffled forms.
18. Hairs on petals	According to visual observations
19. Shape of the base of the petals	From tubular, funnel-shaped, trumpet-shaped, and ligulate.
20. Margin of petals	From entire, serrate, dentate, crenate, and lobed.
21. Venation type	From arcuate, palmate, parallel, pinnate, and reticulate
22. Aestivation type	From valvate, twisted, imbricate, vexillary, and quincuncial.

2.5. Micromorphological Analyses

2.5.1. Stomatal Studies

Leaf samples from *Plumeria pudica* accessions collected across Gujarat were used to assess stomatal characteristics. The abaxial leaf surface was gently washed with distilled water, air-dried, and coated with a thin layer of clear gum, which was allowed to set for 15 minutes. The imprints were removed with transparent adhesive tape and mounted on glass slides for observation under an optical microscope. Stomatal counts were recorded at 10× magnification, while stomatal types were determined at 20× magnification (Herrera-Martínez *et al.*, 2015). Each measurement was replicated four times across three independent samples to minimize error. The stomatal index (SI) was calculated as:

$$SI = \frac{S}{S + E} \times 100$$

where *S* is the number of stomata and *E* is the number of epidermal cells (Aono *et al.*, 2021).

Stomatal density (SD) was determined using a calibrated ocular micrometer (OM) against a stage micrometer (SM). The SM was standardized such that 10 divisions corresponded to 1 mm (0.1 mm or 100 μm per division). Following calibration, the number of stomata in the field of view at 10× magnification was counted, and the field area was calculated as:

$$A = \pi r^2$$

where r is the field radius. Stomatal density was then expressed as the number of stomata per mm^2 of the abaxial leaf surface (Paul *et al.*, 2017).

2.6. Scanning Electron Microscopy (SEM)

Fresh samples of floral and vegetative organs were selected for micromorphological characterization. Plant parts (petals, pollen, leaves, stems, and roots) were affixed to aluminium stubs using double-sided conductive carbon tape to ensure grounding and minimize charging artefacts (University of Nevada, Las Vegas). Samples were cleaned and air-dried before mounting to reduce loose debris (Borisovs *et al.*, 2025). Specimens were sputter-coated with a ~ 10 nm layer of gold (Au) for one minute using a sputter coater. Gold was selected due to its high conductivity, inertness, and fine grain size, which enhance image resolution and minimize charging (Element Pi, 2023; Thermo Fisher Scientific, 2018). A conductive bridge (carbon tape or silver paint) was applied to maintain electrical continuity between the specimen and holder (Charlotte SEM Facility, 2008). Imaging was carried out using a field-emission scanning electron microscope (FE-SEM) at magnifications ranging from $100\times$ to $9000\times$. This range provided sufficient resolution for detailed visualization of fine surface features while reducing beam-induced artefacts (Element Pi, 2023). Structures examined included:

- Anther and Pollen: size, shape, and exine ornamentation.
- Petals: epidermal cell architecture, cuticular striations, and papillate outgrowths.
- Leaves and Stomata: stomatal distribution, morphology, and epidermal surface patterns.
- Stem Epidermis: surface texture and cuticular modifications.
- Roots: presence of root hairs and colonization by arbuscular mycorrhizal hyphae.

2.7. Statical Analysis

Hierarchical agglomerative clustering was employed to explore the similarity structure among morphological and micromorphological traits. Principal Component Analysis was applied to reduce dimensionality and identify the major axes of variation in quantitative morphological traits across the 17 accessions. PCA was conducted on the covariance matrix, and principal components were extracted based on eigenvalues > 1 and scree plot evaluation. All multivariate analyses, including dendrogram visualization from hierarchical clustering and biplot generation from PCA, were performed in R (latest version, RStudio environment), with graphical enhancements applied to improve clarity and interpretability of trait contributions, clustering patterns, and functional groupings.

RESULTS and DISCUSSION

3.1. Quantitative Morphological analysis

A comprehensive morphological evaluation of seventeen *P. pudica* specimens (PP1–PP17) revealed notable variability in both vegetative and reproductive traits (Figure 1). The plants exhibited a wide range of growth patterns, leaf dimensions, and floral structures, reflecting their phenotypic plasticity under observational conditions (Table 3). Quantitative traits were recorded with standard deviations (\pm) to capture intra-plant variability, and the compiled data serve as a baseline for identifying superior genotypes. The specimens varied considerably in height, ranging from 122 ± 2.5 cm in PP2 to an exceptional 579 ± 4.3 cm in PP16. Intermediate values were observed in PP1 (141 ± 3 cm), PP4 (137 ± 4 cm), PP7 (189 ± 2.2 cm), PP10 (236 ± 2.1 cm), and PP14 (292 ± 0.9 cm), with PP15 (548 ± 5.3 cm) and PP17 (335 ± 2.5 cm) representing other tall genotypes. Branching also showed marked differences, with PP16 having only two primary branches but developing the highest number of branchlets (29), while

PP12 exhibited 25 branchlets and PP13 produced 21. By contrast, plants such as PP2 and PP8 displayed reduced branching with only three to four branchlets.

Leaf number and dimensions further highlighted intra-species diversity. PP16 supported the highest leaf count (695 ± 11), followed by PP10 (427 ± 7) and PP15 (421 ± 7), whereas PP11 had the fewest leaves (91 ± 2). Leaf size also varied, with PP16 bearing the largest leaves (30.6 ± 0.26 cm long, 7.7 ± 0.07 cm wide), while PP5 and PP9 produced comparatively smaller leaves around 21–22 cm in length. Leaf petioles ranged from as short as 0.9 cm in PP13 to as long as 2.1 cm in PP16, and leaf thickness generally averaged 0.2–0.4 cm across specimens.

Reproductive traits were equally diverse. The number of inflorescences per plant ranged from as few as 2 in PP2 to as many as 39 in PP16, followed closely by PP15 with 28 and PP17 with 24. Most plants produced inflorescences bearing 15–19 flowers, though slight variations were recorded: PP1 produced 15 ± 3 flowers per inflorescence, PP8 had 13 ± 2 , and PP10 and PP12 each reached up to 19. Flower size also varied considerably, with the smallest flowers (5.3 ± 0.4 cm) observed in PP2 and the largest (9.1 ± 0.4 cm) in PP10. Pedicle length ranged from 0.8 cm in PP2 to 2.1 cm in PP17, while the floral eye zone diameter extended from 1.1 cm in PP2 to 2.5 cm in PP16.

Petal morphology revealed additional variation, with petal lengths ranging between 2.1 cm in PP13 and 3.3 cm in PP10, and widths spanning from 1.2 cm in PP2 and PP13 to nearly 1.9 cm in PP12, PP14, and PP15. Particularly robust floral structures were noted in PP7, PP14, PP15, and PP16, which consistently displayed larger floral dimensions and well-developed petals, contributing to enhanced ornamental appeal. In contrast, accessions with comparatively smaller petals exhibited relatively compact floral architecture, suggesting potential differences in resource allocation between vegetative and reproductive growth.

PP16 stood out as the most vigorous genotype, with exceptional vegetative and reproductive growth, including the tallest height, highest leaf count, and largest number of inflorescences. PP15 and PP10 also demonstrated strong performance in height, foliage, and floral size, whereas PP2 and PP8 represented the more compact phenotypes, with reduced branching and lower reproductive output. These observations underline the influence of genetic variation on the morphological traits of *P. pudica* and provide a valuable foundation for selecting promising genotypes in future studies.

Table 3. Variations in quantitative characters studied on 17 abscissions of *P. pudica*

AB	PL	N B	NB L	NL	ALL	ALW	LP	AL T	NI	NFP	LF	LFP	FED	LP	WP
PP1	141±3	6	6	146±7	25.3±0.21	7.1±0.05	1.8	0.2	4	15±3	6.7±0.3	1.1±0.1	1.5±0.02	2.4±0.03	1.4±0.02
PP2	122±2.5	4	4	124±5	25.5±0.12	6.8±0.09	1.6	0.2	2	17±2	5.3±0.4	0.8±0.05	1.1±0.1	2.1±0.02	1.2±0.03
PP3	128±1.8	4	8	158±3	23.1±0.31	7.5±0.10	1.1	0.3	3	14±2	5.6±0.2	0.9±0.03	1.2±0.03	2.2±0.03	1.3±0.05
PP4	137±4	4	10	187±4	25.6±0.09	6.8±0.07	1.2	0.3	8	15±3	7.4±0.3	1.5±0.2	1.6±0.04	2.8±0.06	1.6±0.03
PP5	138±3.1	3	12	211±5	21.5±0.14	7.1±0.08	1.1	0.2	9	17±3	7.5±0.4	1.3±0.3	1.6±0.03	2.8±0.02	1.7±0.04
PP6	135±4.5	5	15	239±2	25.2±0.10	7.6±0.11	1.4	0.3	7	16±2	6.5±0.3	1.4±0.3	1.3±0.05	2.3±0.05	1.3±0.03
PP7	189±2.2	4	9	171±3	22.3±0.21	6.9±0.06	1.1	0.2	11	17±3	8±0.5	1.2±0.2	2.1±0.06	2.9±0.04	1.7±0.05
PP8	123±2	3	3	93±2	24.2±0.11	7.4±0.07	1.2	0.3	4	13±2	7.7±0.2	1.5±0.1	1.9±0.03	2.6±0.07	1.5±0.02
PP9	134±3.3	3	11	323±8	21.9±0.08	5.8±0.05	1.7	0.2	18	13±2	7.5±0.3	1.3±0.05	1.5±0.02	2.6±0.04	1.5±0.04
PP10	236±2.1	3	13	427±7	28.5±0.17	7.5±0.08	1.7	0.3	16	19±4	9.1±0.4	1.9±0.2	2.4±0.05	3.3±0.07	1.8±0.03
PP11	193±0.5	4	17	91±2	27.4±0.13	8.3±0.09	1.4	0.2	5	18±2	5.9±0.3	0.9±0.05	1.3±0.1	2.4±0.05	1.3±0.02
PP12	224±1	4	25	231±4	25.3±0.11	8.1±0.06	1.9	0.4	9	19±3	7.9±0.2	1.7±0.02	1.7±0.03	2.8±0.02	1.8±0.04
PP13	195±1.5	5	21	354±7	22.5±0.15	6.9±0.04	0.9	0.2	18	17±2	6.1±0.3	1.1±0.02	1.4±0.02	2.1±0.03	1.2±0.03
PP14	292±0.9	4	19	289±6	22.7±0.05	7.1±0.05	1.3	0.3	13	18±3	8.3±0.4	1.8±0.05	2.2±0.06	3.1±0.06	1.8±0.05
PP15	548±5.3	3	18	421±7	28.6±0.21	7.9±0.08	1.7	0.4	28	17±2	8.5±0.2	1.7±0.2	2.1±0.02	3.2±0.03	1.9±0.02
PP16	579±4.3	2	29	695±11	30.6±0.26	7.7±0.07	2.1	0.4	39	18±3	8.4±0.5	1.9±0.03	2.5±0.03	3.1±0.04	1.7±0.04
PP17	335±2.5	3	15	321±7	23.1±0.21	7.5±0.08	1.4	0.3	24	19	7.7±0.3	2.1±0.02	1.9±0.06	2.8±0.03	1.7±0.03

Description: AB: Abscissions; PL: Plant length(cm); NB: Number of branches, NBL: Number of branchlets; NL: Number of leaves per plant; ALL: Average leaf length(cm); ALW: Average leaf width(cm); LP: Length of petiole; ALT: Average leaf thickness; NI: Number of inflorescences per plant; NFP: Number of flowers per plant; LF: length of flower; LFP: Length of flower pedicle; FED: Flower eye zone diameter; LP: Length of petal and WP: Width of petal





Figure 1. Morphological variation in terms of leaf colors; sizes; and shapes among the 17 populations of *P. pudica*

3.1.1. Diversity among Quantitative Morphological Characters

Significant variation was observed among the quantitative morphological characteristics of the plant. This variation could be attributed to the fact that 17 samples were collected from various locations across Gujarat. To quantify the diversity in statistical terms, the calculations of the Simpson's and Shannon-Wiener indices and Pielou's index for each quantitative morphological trait of the plant were done.

3.1.1.1. Simpson's Index

The morphological character analysis revealed considerable diversity across different plant traits, as indicated by the Simpson diversity index (1-D). The number of branches showed the highest diversity (0.934), followed closely by the average length of flowers (0.924) and the number of inflorescences per plant (0.920), suggesting substantial variability in these characteristics. Other floral traits, including the length of flower pedicles (0.897), petal length (0.891), flower eye zone diameter (0.882), and petal width (0.787), also exhibited high diversity values. Vegetative traits demonstrated varying levels of diversity, with leaf width (0.886) and petiole length (0.876) showing particularly high variability, while average leaf length (0.827) and number of leaves per plant (0.796) displayed moderate diversity. Plant height showed intermediate diversity (0.699). The high diversity observed in most traits suggests a genetically diverse population with substantial morphological variation, particularly in floral and leaf characteristics (Figure 2).

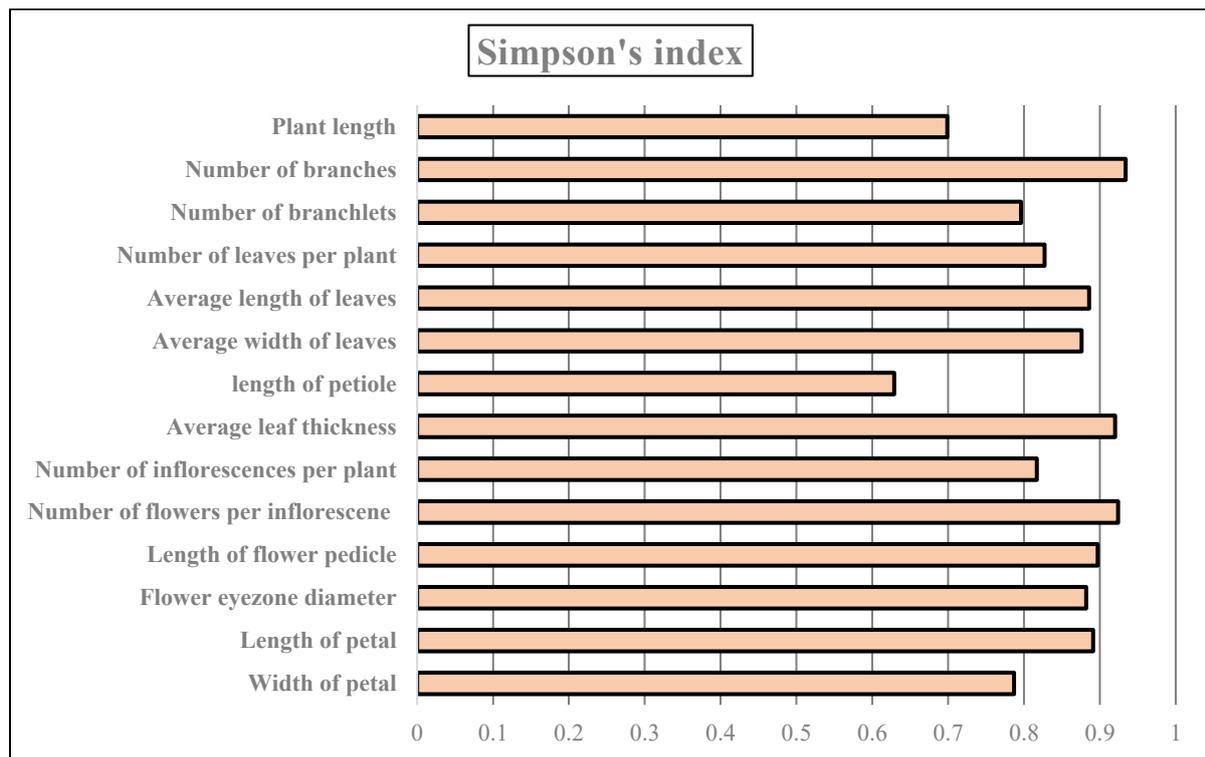


Figure 2. Simpson index for 17 abscissions of *P. pudica*

3.1.1.2. Shannon Wiener's index

The Shannon-Wiener diversity index analysis revealed substantial variation across different morphological characters of the studied plants (Figure 3). The highest diversity was observed in leaf length ($H'=2.91$), followed by branchlet number ($H'=2.72$) and leaf number per plant ($H'=2.65$), indicating these traits exhibited the greatest variability within the population. Floral characteristics showed consistently high diversity values, with flower length ($H'=2.45$), pedicle length ($H'=2.38$), eye zone diameter ($H'=2.42$), and petal dimensions (length $H'=2.41$, width $H'=2.08$) all demonstrating significant variation. Vegetative traits displayed a wider range of

diversity, from relatively low values in leaf thickness ($H'=1.52$) and branch number ($H'=1.84$) to moderate diversity in petiole length ($H'=2.12$) and leaf width ($H'=2.45$). The number of flowers per inflorescence showed the lowest diversity ($H' = 1.27$), suggesting this trait was more uniform across individuals.

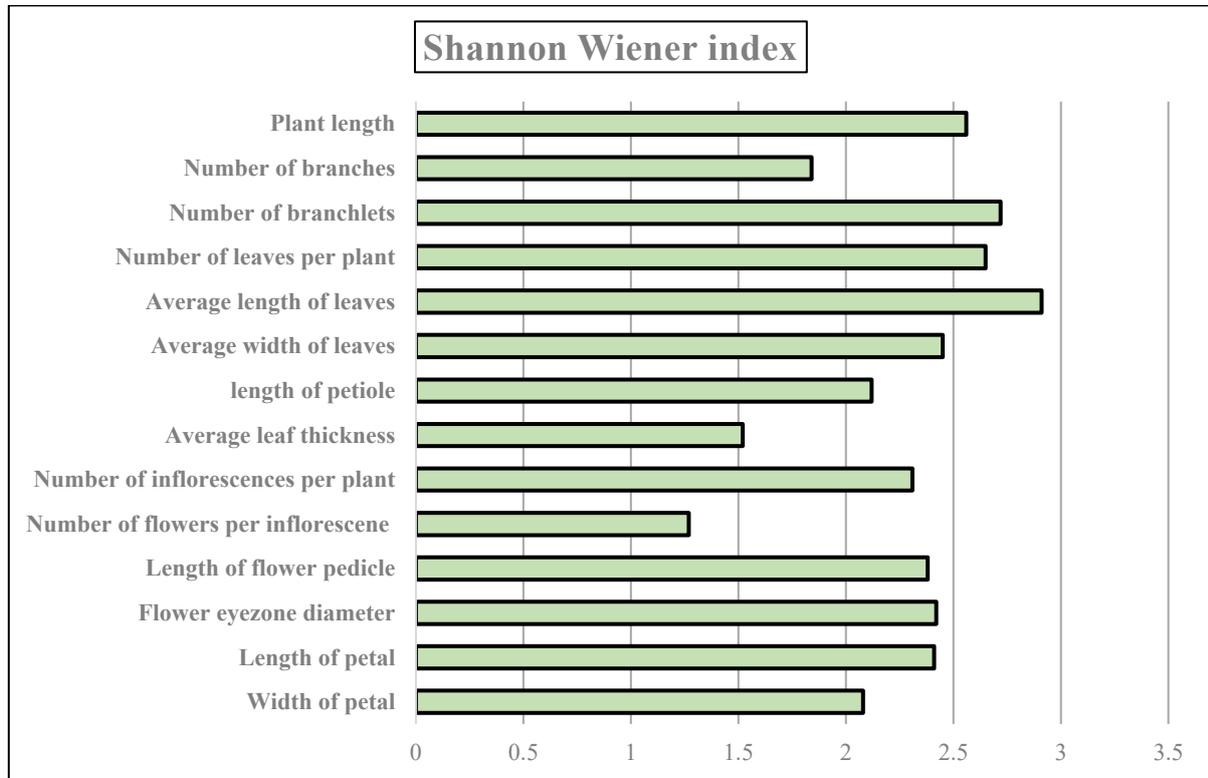


Figure 3. Shannon-Wiener's diversity index for 17 abscissions of *P. pudica*

3.1.1.3. Pielou's index for evenness

Pielou's evenness index values revealed interesting patterns in the distribution of morphological traits across the plant population. Leaf thickness showed near-perfect evenness ($J'=0.96$), indicating an exceptionally uniform distribution of this characteristic, followed closely by plant height ($J'=0.88$) and leaf width ($J'=0.88$), which also exhibited highly equitable distributions. Petiole length demonstrated relatively high evenness ($J'=0.85$), while branch number ($J'=0.80$) and branchlet number ($J'=0.77$) showed moderately even distributions. Floral traits generally displayed slightly lower evenness values, with flower pedicle length ($J'=0.74$), petal length ($J'=0.74$), and eye zone diameter ($J'=0.75$) showing similar distribution patterns. Reproductive structures showed the least even distributions, particularly the number of flowers per inflorescence ($J'=0.65$) and inflorescences per plant ($J'=0.72$). The number of leaves per plant showed the lowest evenness among vegetative traits ($J'=0.68$), suggesting a more clustered distribution.

3.1.2. Variations among quantitative morphological characters

3.1.2.1. PCA analysis

PCA of 17 *P. pudica* abscissions revealed that PC1 explained 55.1% and PC2 13.7% of the total variance, together capturing most of the variation. PC1 axis reflected the overall size and reproductive investment. High-scoring individuals (PP10, PP14–PP17) were tall, highly branched, and produced numerous leaves and flowers. PP16 was the most vigorous (579 cm, 29 branches, 695 leaves, 39 inflorescences, 18 flowers), while PP10 stood out for its flower abundance (427, longest 9.1 cm). In contrast, low-scoring abscissions (PP1–PP9, PP11, PP13) were shorter, less branched, and weakly reproductive (Figure 4). For example, PP2–PP3 (122–128 cm) were compact with minimal flowering, while PP11 was tall (193 cm) but

leaf-poor (91). PC2 axis captured differences in leaf and flower dimensions. High-scoring individuals (PP6, PP11, PP12, PP16) had larger leaves and floral parts, e.g., PP12 with broad petals, PP11 with long flowers despite few leaves. Low scorers (PP4, PP5, PP7, PP9, PP14, PP17) had smaller organs, though some (PP14) still produced many leaves. PC1 separated vigorous, highly reproductive plants from smaller, low-yielding ones. PC2 distinguished individuals with large versus small leaf and floral traits. PP16 emerged as the most vigorous outlier, while PP2–PP3 were the least developed. PP11 showed unusual allocation (tall but leaf-poor, long flowers). Overall, PCA highlighted vegetative vigor and reproductive allocation as the dominant axes of variation in *P. pudica*.

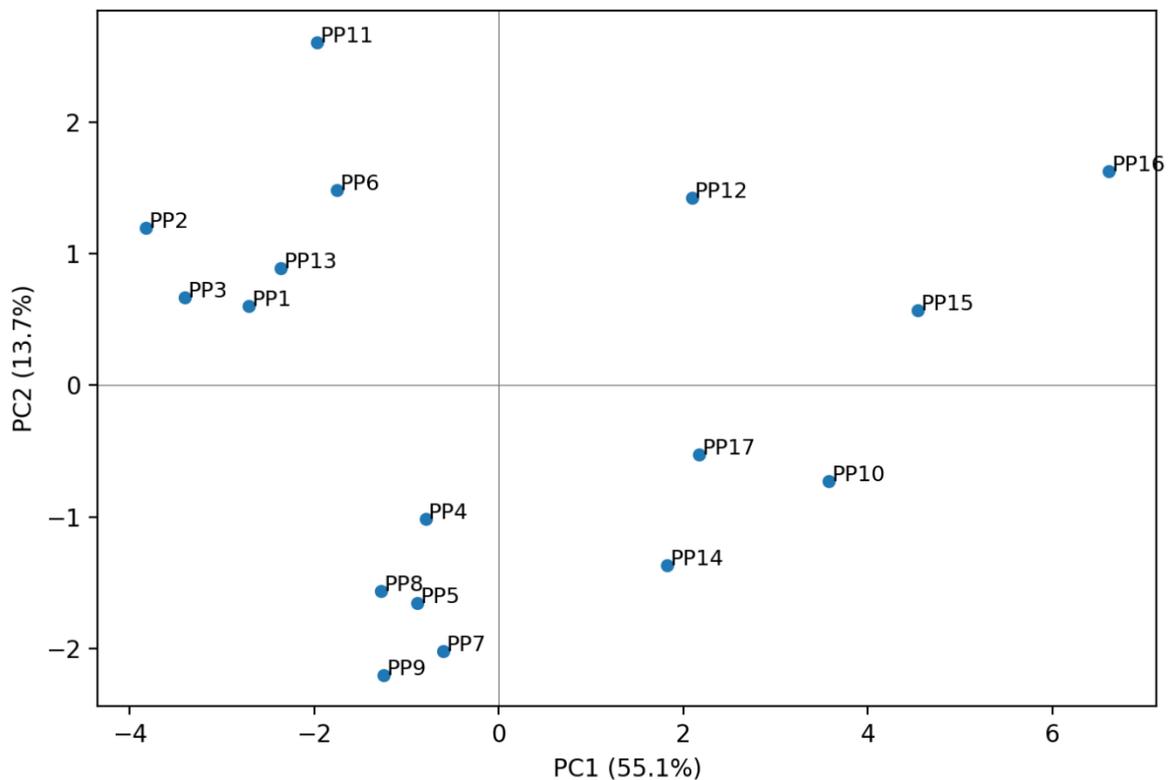


Figure 4. PCA biplot of Quantitative morphological characters of 17 abscissions of *P. pudica*

3.1.2.2. Correlation analysis

The correlation heatmap of quantitative traits in *P. pudica* across 17 abscissions revealed several key patterns in vegetative and reproductive development (Figure 5). The strong positive correlations were observed among traits associated with floral structure, including the length of flower (LF), length of petal (LP2), flower eye zone diameter (FED), and width of petal (WP), indicating tightly coordinated floral development. Notably, LF and LP2 showed a high correlation of 0.95, while FED and LP2 correlated at 0.91, and LF and WP at 0.93. Among vegetative traits, the number of leaves (NL) and the number of inflorescences (NI) exhibited a very strong correlation of 0.93, suggesting that plants producing more leaves tend to also bear more inflorescences. Additionally, plant length (PL) showed a strong association with NI at 0.88, reinforcing the link between overall plant vigor and reproductive output. Moderate correlations were observed between traits such as average leaf length (ALL) and petiole length (LP) at 0.68, reflecting integrated vegetative growth patterns. In contrast, several traits exhibited negative correlations, such as between NI and the number of branches (NB) at -0.59, and between PL and NB at -0.53, indicating potential trade-offs in resource allocation. These patterns suggested the existence of three functional trait groupings: vegetative traits (PL, NL, NB), reproductive traits (LF, FED, LP2), and intermediate traits like average leaf thickness (ALT), which correlated moderately with both vegetative and floral traits.

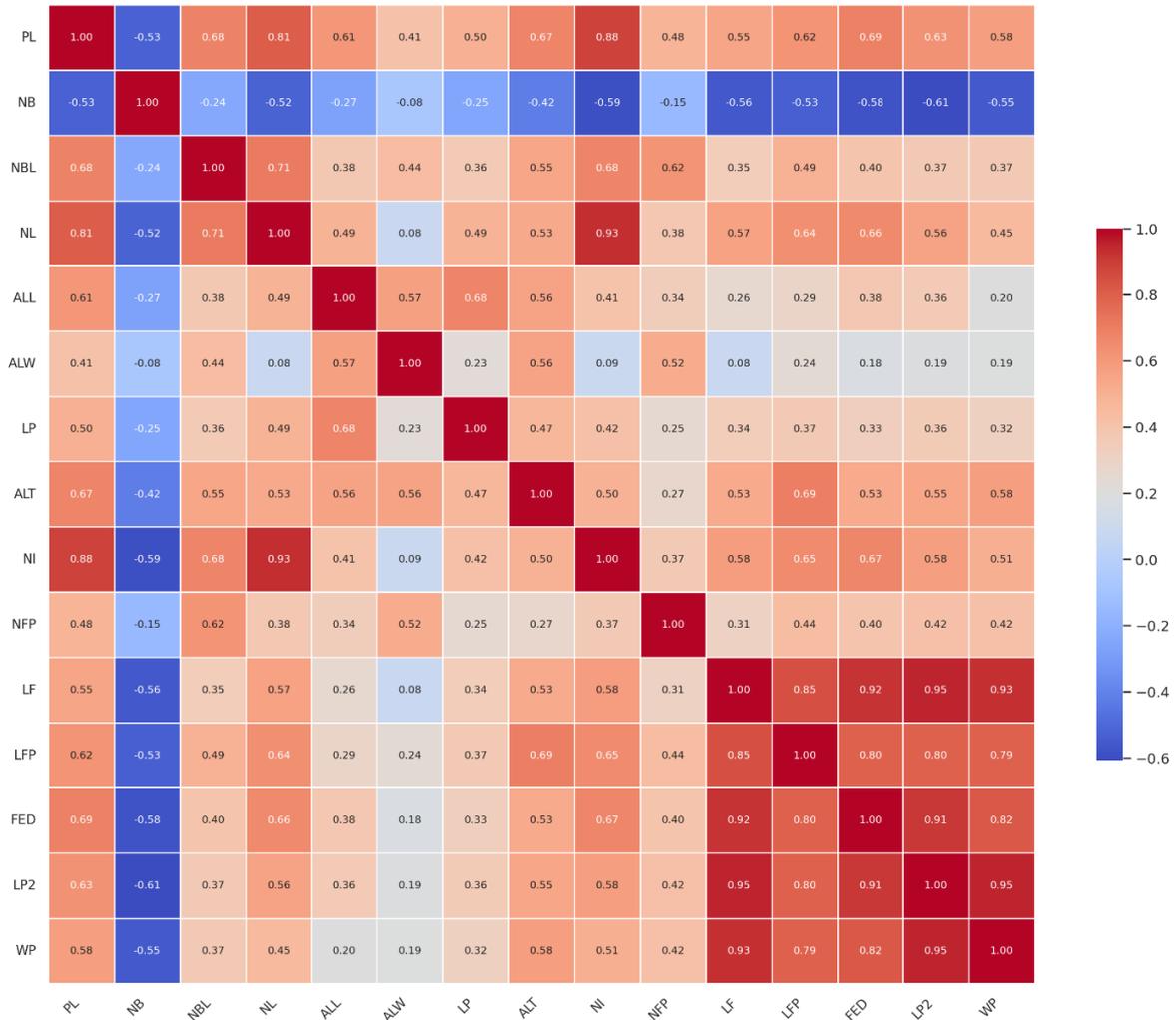


Figure 5. Correlation Among Quantitative Morphological Characters for *P. pudica*

3.1.2.3. Hierarchical clustering

The dendrogram presented the results of hierarchical clustering based on correlation distances among the quantitative traits of *P. pudica*, revealing how various traits were interrelated in terms of their developmental and functional roles (Figure 6). The traits with higher absolute correlations were grouped and connected at lower linkage distances, which suggested close associations. One prominent cluster comprised floral structure traits such as Length of Flower (LF), Length of Petal (LP2), Width of Petal (WP), and Flower Eye Zone Diameter (FED), highlighting their strong correlations and suggesting that these floral dimensions developed in a coordinated and integrated manner. Another well-defined cluster included Plant Length (PL), Number of Leaves (NL), Number of Inflorescences (NI), and Number of Branchlets (NBL), reflecting a strong vegetative-reproductive linkage and emphasizing their collective contribution to plant growth and reproductive potential. Additionally, Average Leaf Length (ALL) and Length of Petiole (LP) were mentioned as forming a distinct sub-cluster, likely due to their structural and functional relationship within leaf morphology. Certain traits, such as Average Leaf Thickness (ALT) and Length of Flower Pedicle (LFP), were noted to occupy intermediate positions in the clustering system, suggesting they played a supportive yet non-central role across trait groupings. Conversely, traits like Number of Branches (NB), Number of Flowers (NFP), and Average Leaf Width (ALW) appeared more isolated, indicating specialized functions or independent variation relative to the broader trait structure.

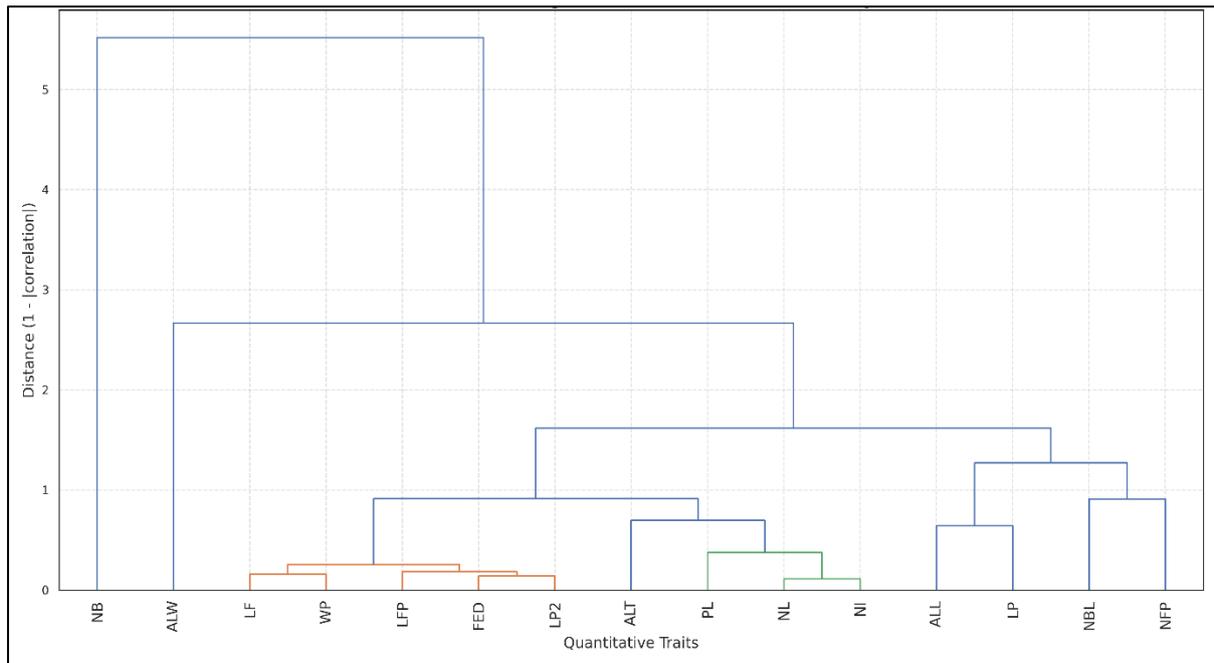


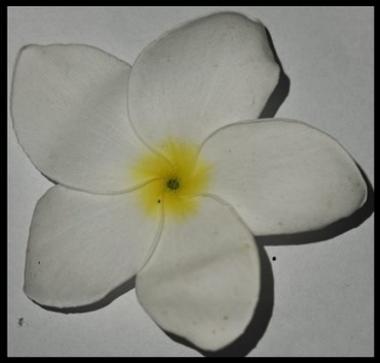
Figure 6. Dendrogram Clustering for Quantitative Characters of *P. pudica*

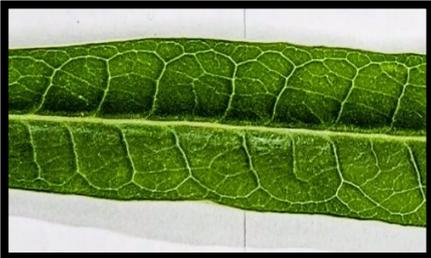
3.2. Qualitative morphological analysis

P. pudica is a woody perennial shrub that typically grows up to 12 feet in height. However, some specimens may exceed this limit due to their upright growth habit, while still maintaining a limited canopy spread that prevents them from being classified as trees. The stems are smooth but fragile, and the leaves, arranged acropetally, vary in both shape and color, ranging from fiddle-shaped to cuneate and pointed forms, with shades from dark green to lighter tones (Table 4). Each leaf has an attenuate base tapering towards the petiole and an aristate apex extending into a sharp awn. Microscopic observations revealed anisocytic stomata on the abaxial surface, with three subsidiary cells surrounding the guard cells, while the adaxial surface displayed a reticulate venation pattern. The inflorescences occur in umbellate clusters with multiple flowers emerging from a central point, and each flower shows twisted aestivation with spirally overlapping petals. The flowers are predominantly white with yellowish to greenish eye zones, borne on receptacles ranging from yellow to parrot green, while the corolla is tubular with circular to oblong petals that enhance the overall aesthetic appeal of the species.

Table 4. Qualitative morphological analysis for *P. pudica*

No.	Morphological trait	Observation	Photograph
1.	Type of plant	Shrub	
2.	Plant growth habit	Upright	
3.	Leaf color	Dark green	
4.	Leaf growth on the stem	Acropetal	
5.	Texture of the leaf	Smooth	
6.	Leaf shape	Fiddle shaped	
7.	Type of stomata	Anisocytic stomata (abaxial surface)	

8.	Leaf base shape	Attenuate leaf base	
9.	Leaf apex shape	Aristate leaf apex	
10.	Type of inflorescence	Umbel inflorescence	
11.	Petal arrangement on flowers	Twisted arrangement	
12.	The color of the flower	White	
13.	Color of the flower's eye zone	From yellow to light green	
14.	Type of aestivation	Twisted	
15.	Color of the receptacle	Yellow to parrot green	
16.	Shape of petals		
17.	Hairs on petals	Absent	
18.	Corolla shape	Tubular	

19.	Margin of petals	Rounded	
20.	Type of venation	Reticulate	

3.3. Electron microscopic study

With the usage of Scanning electron microscopy, the external surfaces of various parts (leaf, stem, root, petals, anthers, and petals) of *P. pudica* were studied for evaluation of external cellular natalities of the plant.

3.3.1. Anther

The high-resolution scanning electron microscope (SEM) micrograph of the *P. pudica* anther revealed several distinct structural characteristics of its pollen grains, confirming their identity and developmental status. It was noted that the pollen grains were predominantly spheroidal to subprolate in shape and exhibited uniform size and morphology, indicating well-developed and viable pollen (Figure 7). Additionally, the surface of the pollen was described as finely textured with a reticulate exine ornamentation, characterized by a net-like pattern formed by shallow ridges and polygonal depressions. This sculpturing was identified as a hallmark of entomophilous (insect-pollinated) species, enhancing adherence to pollinators during pollen transfer. It was also observed that while the tricolpate nature, which consists of three furrow like apertures typical of Apocynaceae pollen, was not visible in the micrograph.

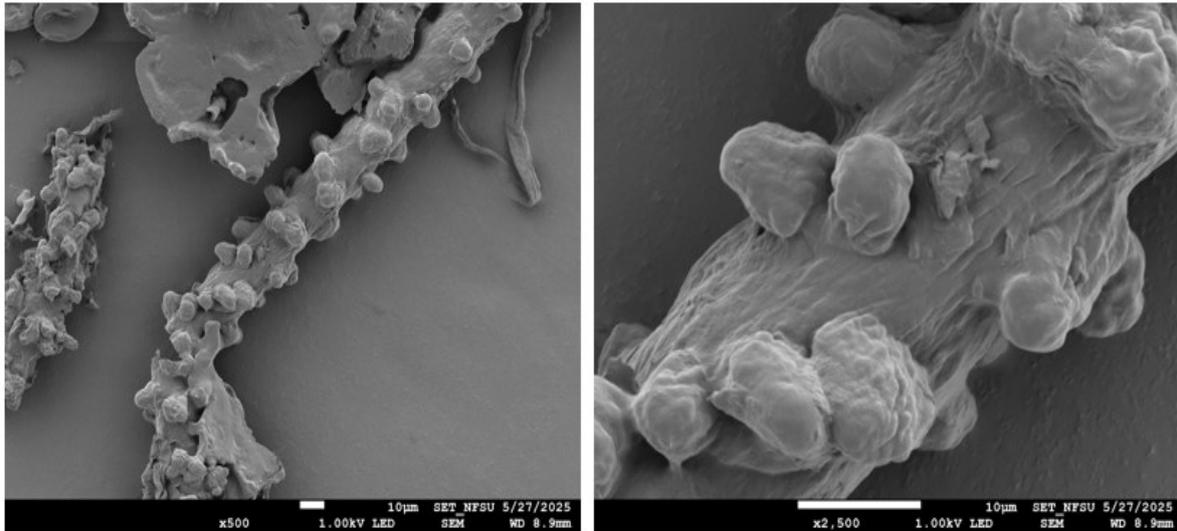


Figure 7. SEM photographs of anthers, where slide 1: Visualisation of an anther at 500x resolution; and slide 2: Visualisation of an anther at 2500x resolution

3.3.2. Petal

The scanning electron microscopy (SEM) analysis of *P. pudica* petals revealed highly detailed cellular formations that reflected both structural complexity and potential functions of the corolla. A prominent feature observed was the epidermal cell architecture, which consisted of polygonal to irregularly shaped cells exhibiting well-defined, slightly undulating anticlinal walls. This cell shape arrangement is typical of adaxial petal surfaces in many angiosperms, particularly in those adapted for ornamental and pollinator-attracting functions. The undulating anticlinal boundaries contributed to the optical reflectance and light scattering, thereby intensifying petal coloration. These cuticular features are produced by the deposition of cutin and waxy substances, which serve several biological functions. Certain regions of the images also suggested the presence of dome-shaped or papillate outgrowths, elevated convex structures that may play roles in light manipulation and water repellence. Importantly, no glandular or non-glandular trichomes or stomata were observed in the SEM fields examined (Figure 8). The petals also exhibited exceptional surface cleanliness and integrity. The absence of physical particulate residues indicates that the sample preparation method was effective in preserving delicate petal morphology. The natural waxy cuticle, clearly visible under SEM, likely contributed to this preservation during the dehydration and gold-coating processes commonly employed in SEM sample preparation.

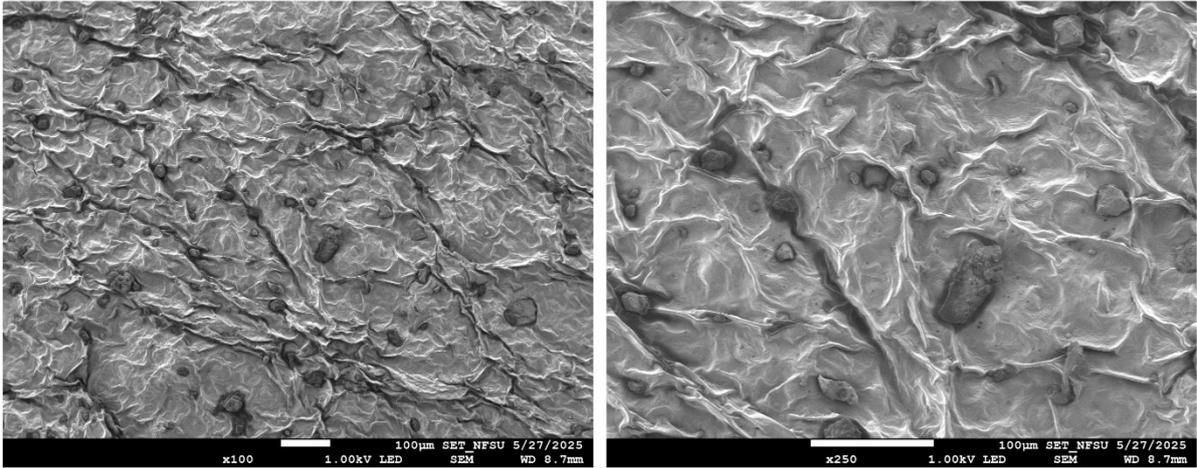


Figure 8. SEM photographs of petals, where slide 1: Visualisation of a petal at 100x resolution; and slide 2: Visualisation of a petal at 250x resolution

3.3.3. Leaf and stomata

The scanning electron micrographs (SEM) of the leaf surface of *P. pudica* revealed intricate details of both stomatal and epidermal structures of the leaf, reflecting the plant's adaptation to its environment. The stomata were visible, comprising pairs of prominent kidney-shaped guard cells that enclosed a central pore. These stomatal pores appeared elliptical to slightly open, likely reflecting the physiological state of the tissue during fixation. The stomata were positioned either flush with or slightly sunken into the surrounding epidermis, a structural feature that may help reduce excessive water loss through transpiration. Their distribution across the surface suggested a hypostomatic condition, meaning the stomata were localized primarily on the abaxial (lower) leaf surface. The surrounding subsidiary cells did not follow a consistent radial arrangement (Figure 9). In addition to the stomata, the general leaf surface architecture showcased a well-organized epidermal layer composed of polygonal to irregular cells with distinct, wavy anticlinal walls. These epidermal cells were tightly interlocked, forming a mechanically robust and continuous surface. Notably, the surface was completely glabrous, with no evidence of trichomes or epidermal hairs in the scanned areas. A thin but distinct cuticular layer coated the epidermis, exhibiting fine granulation and subtle striations representing wax deposition.

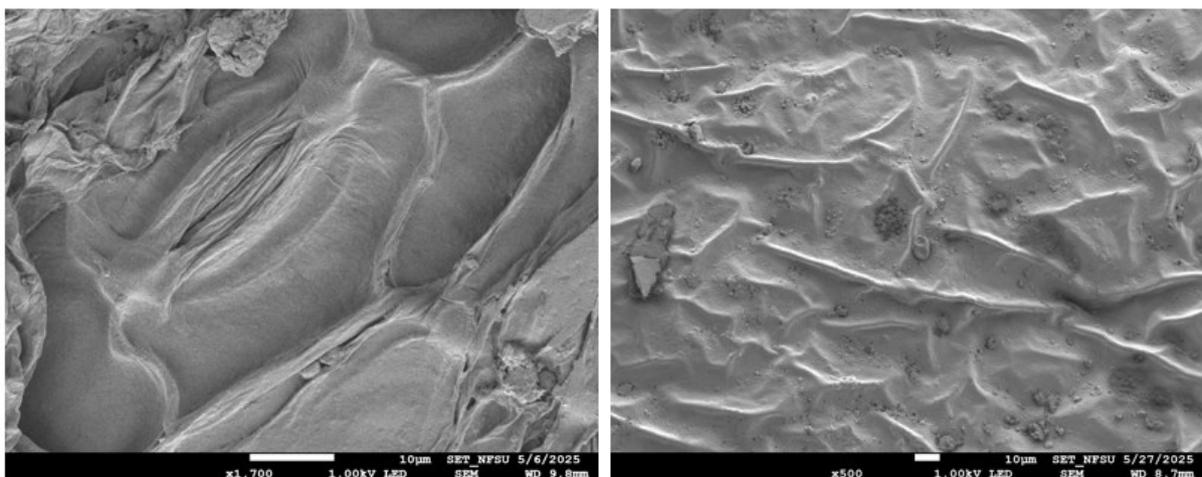


Figure 9. SEM photographs of anthers, where slide 1: Visualisation of stomata at 1700x resolution; and slide 2: Visualisation of leaf surface at 500x resolution

3.3.4. Stem

The scanning electron micrographs (SEM) of the stem surface of *P. pudica* revealed a well-organized and intact outer epidermal layer composed of polygonal to irregularly shaped cells. These epidermal cells were compactly arranged with clearly defined boundaries, forming a continuous, protective surface. The shape and size of the cells varied slightly but generally remained isodiametric, with no significant elongation, indicating a typical non-specialized epidermis associated with mature stem regions. A notable feature observed across the images is the presence of cuticular ornamentation in the form of subtle ridges and surface granulations. These surface textures likely arise from cutinized or waxy deposits that form part of the outer cuticle, which serves several critical functions (Figure 10). In some regions, the surface displayed a granular or slightly striated appearance, possibly due to epicuticular wax crystals or secreted exudates that have dried on the surface. Interestingly, no trichomes, either glandular or non-glandular, were visible in the examined regions. This confirmed the glabrous nature of *P. pudica* stems, which are characteristically smooth and shiny.

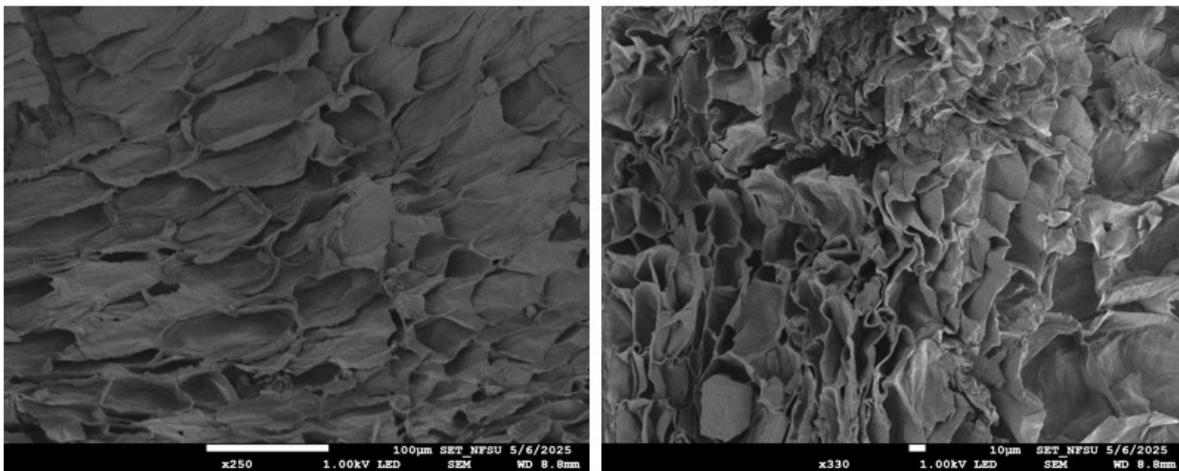


Figure 10. SEM photographs of the stem surface, where slide 1: Visualisation of the stem surface at 250x resolution; and slide 2: Visualisation of the stem surface at 330x resolution

3.3.5. Root

The scanning electron micrograph (SEM) of the *P. pudica* root surface revealed a well-organized and intact outermost epidermal layer, known as the rhizodermis. The epidermal cells were polygonal, tightly packed, and uniformly distributed, indicating strong structural integrity and a mature developmental stage of the root. This orderly arrangement reflects minimal physical disruption and healthy root growth. Subtle ridging or surface elevations were visible along the cell walls, which may be attributed to cuticular deposits or natural thickening of the epidermal cell boundaries. A few shallow fissures or cracks appeared across the surface, potentially representing natural zones of expansion during root elongation or early lateral root emergence. A particularly noteworthy observation in this SEM image was the presence of elongated, tubular structures emerging from the rhizodermis. These extensions were smooth-surfaced, tapering toward the end, and varied slightly in length. Their sparse distribution and structural simplicity were consistent with the characteristics of root hairs, especially in maturing zones of the root (Fig. 11). In another region of the root surface, the SEM revealed numerous globular bead-like structures on the epidermal cells. These structures appear non-septate and smooth, which strongly suggests that they were hyphae of arbuscular mycorrhizal (AM) fungi. Their intimate contact with the epidermis and the presence of subtle invaginations indicated possible entry points for fungal penetration into the root cortex, hallmarks of AM fungal colonization. The structurally mature and stable root surface, with its tightly packed epidermal cells and protective ridging, appears well-adapted for mechanical strength and anchorage.

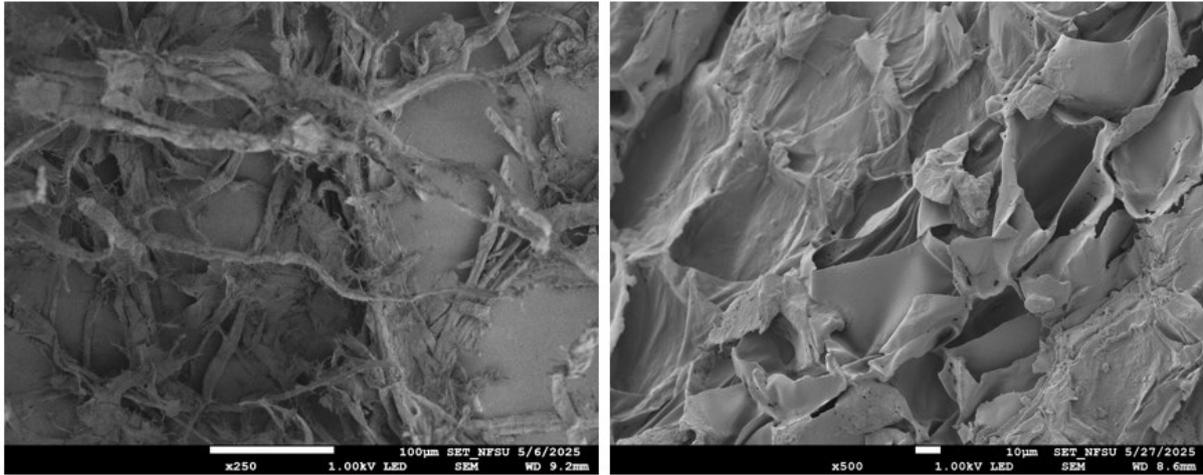


Figure 11. SEM photographs of root surface, where slide 1: Visualisation of root hairs at 250x resolution; and slide 2: Visualisation of root surface at 500x resolution

3.4. Anatomical studies

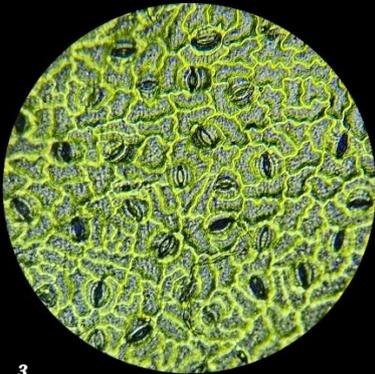
3.4.1. Stomatal studies

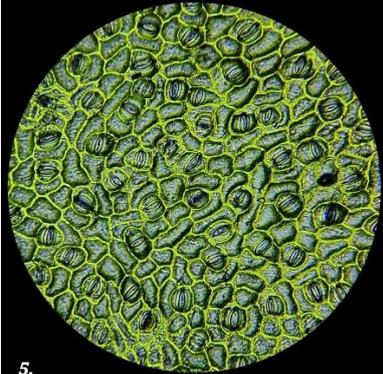
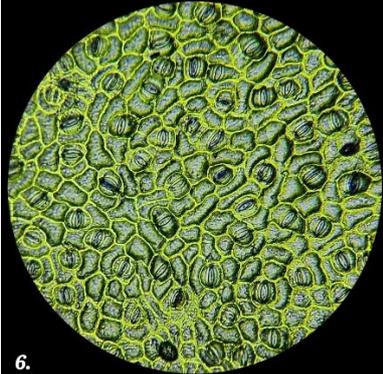
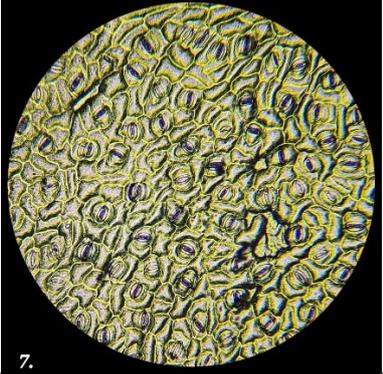
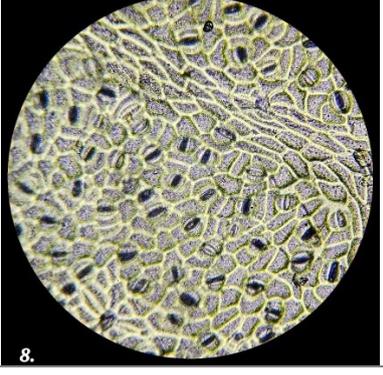
In *P. pudica*, the stomata were observed to have a kidney-like shape and were randomly dispersed on the abaxial surface of the leaves. The density of stomata was found to be highest on this abaxial side, which may help to reduce water loss due to decreased exposure to thermal heating. Furthermore, it was noted that the distribution and patterning of stomata on the lower surface of the leaf seem to be random, with no clear distribution patterns identified.

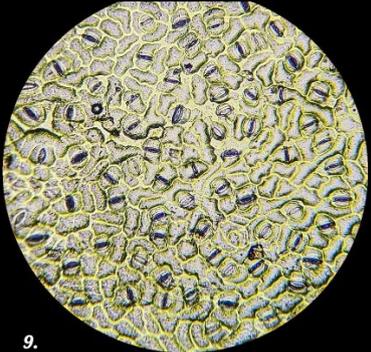
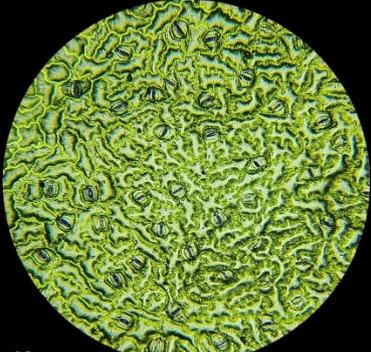
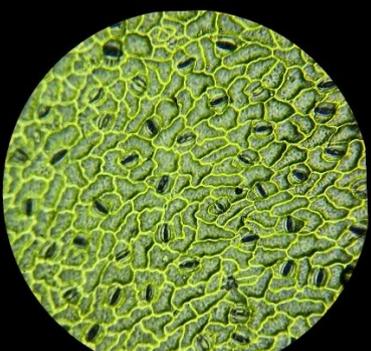
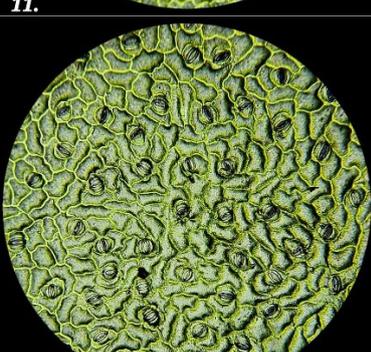
3.4.1.1. Stomatal Index and Stomatal Density

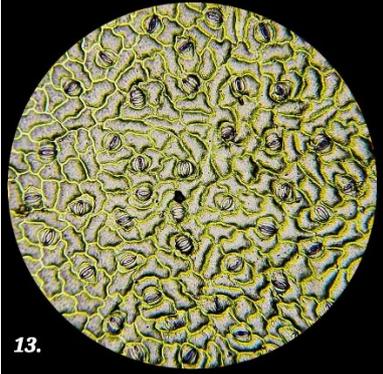
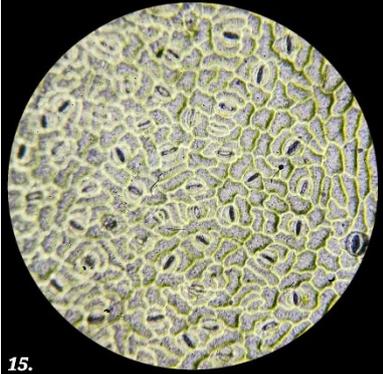
A strategy employed by plants to adapt to prevailing environmental conditions is to modulate the frequency of stomatal development. The study of the stomatal index gave an idea about the *P. pudica*'s relation with the environmental surroundings and its coping mechanism (Table 5). The stomatal characteristics of 17 abscissions from *P. pudica* plants were analyzed, focusing on stomatal index (%) and stomatal density (mm^2). The stomatal index ranged from 23.46% (PP3) to 34.05% (PP14), while stomatal density varied between 29.44 mm^2 (PP15) and 51.28 mm^2 (PP9). Notably high values for both parameters were observed in PP5, PP8, and PP9, indicating increased stomatal activity in those abscissions. Diversity analyses revealed a Simpson's diversity index of 0.767 for the stomatal index and 0.743 for stomatal density, suggesting a relatively high distributional variability among samples. The Shannon-Wiener diversity index values further supported this, with 1.391 for stomatal index and 1.277 for stomatal density, reflecting substantial heterogeneity in stomatal features across the abscissions.

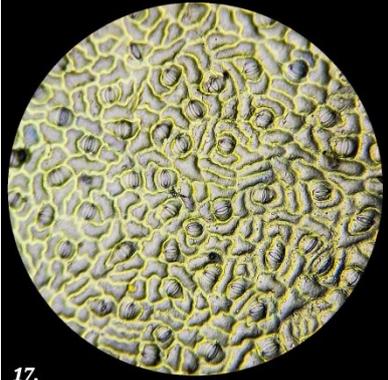
Table 5. Variations in stomatal traits for 17 abscissions of *P. pudica*

Abscission	Stomatal index (%)	Stomatal density (mm ²)	Photograph
PP1	28.82	36.95	 <p>1.</p>
PP2	24.29	32.42	 <p>2.</p>
PP3	23.46	29.51	 <p>3.</p>
PP4	25.58	33.18	 <p>4.</p>

PP5	33.16	49.01	
PP6	31.14	42.98	
PP7	31.48	46.75	
PP8	33.51	46.02	

PP9	33.66	51.28	 9.
PP10	25.44	32.43	 10.
PP11	23.83	30.92	 11.
PP12	26.43	34.69	 12.

PP13	28.34	35.12	 13.
PP14	34.05	35.44	 14.
PP15	24.07	29.44	 15.
PP16	26.84	38.46	 16.

PP17	27.67	36.19	
Simpson's diversity	0.767	0.743	
Shannon Wiener diversity	1.391	1.277	

3.4.1.2. Correlation analysis

For the correlation analysis among the various stomatal traits and morphological traits, it was observed that the plant length showed moderately strong positive correlations with leaf thickness ($r = 0.67$) and leaf length ($r = 0.61$), meaning taller plants tend to have longer and thicker leaves (Fig. 12). Leaf width, however, exhibited a weaker positive correlation with plant length ($r = 0.41$). Additionally, leaf length was positively correlated with both leaf width ($r = 0.57$) and leaf thickness ($r = 0.56$), suggesting that these leaf size dimensions tend to increase together. A very strong positive correlation was observed between stomatal index and stomatal density ($r = 0.85$), indicating that a higher stomatal index is strongly associated with greater stomatal density. On the other hand, the Stomatal index was negatively correlated with leaf length ($r = -0.60$), leaf width ($r = -0.49$), and leaf thickness ($r = -0.28$), with stomatal density showing similar negative trends. This implied a theory that plants with larger or thicker leaves tend to have lower stomatal index and density. Furthermore, plant length has a slight negative correlation with stomatal index ($r = -0.25$) and stomatal density ($r = -0.29$), indicating that taller plants and plants with long and broader leaves have reduced stomatal characteristics.

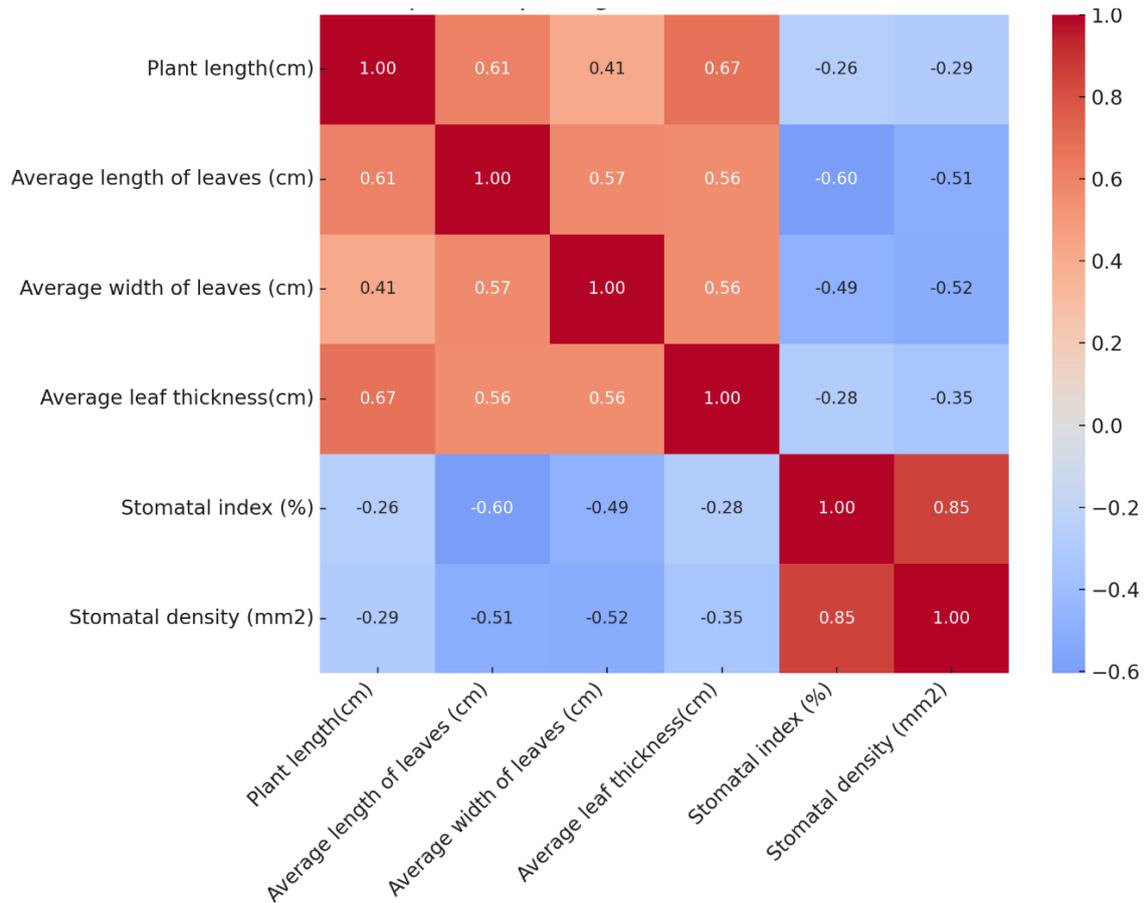


Figure 12. Analysis of the correlation between stomatal characters and morphological characters

CONCLUSION AND RECOMMENDATION

This study of 17 *P. pudica* abscissions across diverse agroclimatic zones of Gujarat revealed pronounced phenotypic variation in both vegetative and reproductive traits. Plant height ranged from 122 ± 2.5 cm to 579 ± 4.3 cm and leaf numbers from 91 ± 2 to 695 ± 11 , alongside variation in leaf dimensions (21.5–30.6 cm length; 5.8–8.3 cm width). Such diversity reflects strong phenotypic plasticity, likely driven by genotype–environment interactions shaped by heterogeneous soils, microclimates, and moisture regimes. Reports across tropical perennials confirm that intraspecific variation is a critical mechanism for coping with habitat heterogeneity, reinforcing similar trends observed here (Westerband *et al.*, 2021; Joshi *et al.*, 2021; Piao, 2014). Our data indicate that *P. pudica* flexibly adjusts leaf and floral dimensions to resource availability and stress factors, highlighting its adaptive capacity, while stability in traits such as leaf thickness and branch number suggests developmental constraints maintain certain conserved features. This balance of trait plasticity and stability equips the species to thrive under variable ecological conditions.

When compared to congeners, *P. pudica* consistently exhibited narrower leaves and shorter petioles relative to *P. obtusa*, features likely reflecting differences in water-use strategies and light interception efficiencies (Fahn, 1990). Floral morphology provided further contrasts: while flower size overlapped with *P. obtusa* and *P. rubra*, *P. pudica* displayed a compact floral design with shorter pedicels, a feature possibly tied to specialized pollination syndromes (Endress & Bruyns, 2000; Zhou *et al.*, 2022). At the micromorphological level, pollen exine ornamentation in *P. pudica* was finely reticulate, contrasting with the smoother, psilate texture of *P. alba*, and its petal epidermis lacked the trichomes often found in other *Plumeria* species (Agustiar *et al.*,

2020). Leaf features also diverged from genera such as *Nerium* and *Catharanthus*, which are trichome-rich and show greater stomatal diversity (Salim *et al.*, 2021). These comparisons underscore how different members of Apocynaceae diverge morphologically to align with their environments.

Trait associations in *P. pudica* were evident in multivariate analyses, with PCA revealing two major axes of variation: one reflecting overall vegetative–reproductive vigor and the other distinguishing patterns in leaf and floral morphology. Vigorous accessions such as PP16 combined tall stature, extensive branching, and prolific flowering, while other accessions represented more constrained growth and reproductive output. These coordinated patterns echo integrated trait modules reported in *P. alba* and *Catharanthus roseus* (Sharma *et al.*, 2013; Yadava *et al.*, 2012), suggesting stable evolutionary regulation of functional complexes. Hormonal dynamics, including auxins and cytokinins, together with source–sink allocation strategies, likely underlie trade-offs between vegetative growth and reproductive output (Taiz *et al.*, 2015; Su *et al.*, 2011). Such integrated development ensures functional balance, maintaining reproductive success across environments.

The observed morphological and micromorphological diversity also carries clear ecological and horticultural implications. Plasticity in branching, flowering, and leaf design confers resilience under abiotic stressors such as drought, nutrient limitation, and light variability, reinforcing the ornamental value of *P. pudica* in landscaping and urban greening. SEM analyses revealed xeromorphic adaptations, including sunken anisocytic stomata restricted to the abaxial surfaces, a reticulate cuticle, and a hypostomatic leaf design that reduces water loss while permitting effective gas exchange. One of the most notable findings of this work is the first evidence of root mycorrhizal association in *P. pudica* visualized via SEM, where hyphae were detected alongside root hairs. This dual system of direct absorption and symbiotic nutrient uptake likely enhances survival in nutrient-poor soils and provides novel insight into the species' ecological strategies. Such an association has not been previously described in *Plumeria* or related Apocynaceae, underscoring the novelty of this result.

Overall, these findings highlight *P. pudica* as a species capable of balancing diverse adaptive processes, from pollinator specialization to drought tolerance and nutrient acquisition, ensuring ecological versatility and horticultural success. Nevertheless, limitations remain, as morphological and SEM analyses alone cannot clarify the underlying genomic and physiological mechanisms that shape these traits. Future studies integrating population-level genomic tools, epigenetic profiling, and physiological experiments will be crucial to unravel the molecular drivers of plasticity and adaptation in *P. pudica*. Such approaches will not only enrich taxonomic and ecological understanding of the genus but also guide selective breeding and conservation strategies for this widely cultivated ornamental. This research provides the first comprehensive integrative morphological and micromorphological evaluation of *Plumeria pudica*, revealing remarkable intraspecific diversity and ecological plasticity across 17 accessions from Gujarat and Daman. The study documents broad variation in plant structure, leaf morphology, and floral traits, shaped by genetic and environmental interactions. SEM-based microstructural analyses uncover distinctive pollen exine ornamentation, petal epidermal patterns, stomatal distribution, and root fungal symbioses, highlighting adaptations that likely contribute to successful pollination, water conservation, and nutrient uptake. Correlative and clustering analyses demonstrate coordinated development of vegetative and reproductive traits, reflecting evolutionary pressures and ecological function. These multidimensional insights enhance our understanding of *P. pudica*'s biology and ecology, laying the groundwork for its conservation, breeding, and utilization as a model ornamental species within the Apocynaceae. Future research integrating molecular genetics and physiological assessments will further enhance understanding of this species' adaptive potential.

Statements and declarations

Data availability

All the data that has been generated during this research are included in the manuscript.

Acknowledgment

We wish to convey our heartfelt thanks to Dr. N.K. Odedra and Dr. A.R. Modhvadiya, associate professors in the Botany Department at M.D. Science College in Porbandar, for their essential guidance and assistance during this research. Additionally, we would like to acknowledge our institute for offering the resources and equipment required for the successful completion of this study.

Research compliance statement

All field studies and experimental research discussed in this article, encompassing the gathering of plant material and laboratory analyses, follow ethical guidelines. These activities conform to the regulations, legislation, and standards established by the institution (M.D. Science College), in addition to national and international laws.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Use of generative AI

Artificial intelligence tools (ChatGPT, OpenAI) were used only for language refinement and grammar improvement. No AI was used for data analysis, interpretation, or drawing conclusions.

Authorship contribution statement

The conceptual framework of this study was developed by KS and BAJ. Data curation and the execution of experiments were exclusively performed by KS. Formal analysis and validation were carried out by BAJ, who also managed the project administration. The development of the methodology was a joint endeavor involving KS, BAJ, and KNO. KS and KNO composed the initial draft of the manuscript. All data were generated internally, and the authors collectively affirm their accountability for all aspects of the work, thereby ensuring its integrity and accuracy.

REFERENCE

- Agustiar, A. B., Masyitoh, D., Fibriana, I. D., Khumairoh, A. S., Rianti, K. A., Fitriani, N., ... & Akmalia, H. A. (2020). Phenetic Kinship Relationship of Apocynaceae Family Based on Morphological and Anatomical Characters. *Bioeduscience*, 4(2), 113-119.
- Barthlott, W., Mail, M., Bhushan, B., & Koch, K. (2019). Plant Surfaces: Structures and Functions for Biomimetic Innovations. *Nano-Micro Letters*, 11(1), 65. <https://doi.org/10.1007/S40820-018-0225-5>
- Beerling, D. J., & Kelly, C. K. (1997). Stomatal Density Responses of Temperate Woodland Plants Over The Past Seven Decades of CO₂ Increase: A Comparison of Salisbury (1927) With Contemporary Data. *American Journal of Botany*, 84(11), 1572-1583.

- Borisovs, V., Bossi, M., Matino, L., Marmioli, P., & Cavaletti, G. (2025). New Approaches Based on Serial-Block Face Electron Microscopy to Investigate the Peripheral Nervous System. *Journal of the Peripheral Nervous System*, 30(2), E70019. <https://doi.org/10.1111/Jns.70019>
- Bossdorf, O., Richards, C. L., & Pigliucci, M. (2008). Epigenetics For Ecologists. *Ecology Letters*, 11(2), 106–115. <https://doi.org/10.1111/J.1461-0248.2007.01130.X>
- Bradshaw, A. D. (1965). Evolutionary Significance of Phenotypic Plasticity in Plants. *Advances in Genetics*, 13, 115–155.
- Carlquist, S., & Schneider, E. L. (2013). Apocynaceae Wood Evolution Matches Key Morphological Innovations. *American Journal of Botany*, 100(5), 851–867. <https://doi.org/10.3732/Ajb.1200548>
- Choubey, A., Kumar, A., Kumar, R., & Singh, R. (2018). Identification of Three Kinds of Plumeria Flowers by DNA Barcoding And Hplc Specific Chromatogram. *Scientific Reports*, 8, 1–10. <https://doi.org/10.1038/S41598-018-27584-5>
- Demarco, D. (2017). Histochemical Analysis of Plant Secretory Structures. In *Histochemistry of Single Molecules: Methods and Protocols* (Pp. 313–330). Springer.
- Diggle, P. K. (2014). Modularity And Integration in Floral Development and Evolution. *International Journal of Plant Sciences*, 175(7), 617–626. <https://doi.org/10.1086/676004>
- Endress, M. E., & Bruyns, P. V. (2000). A Revised Classification of The Apocynaceae S.L. *Annals of the Missouri Botanical Garden*, 87(4), 532–555. <https://doi.org/10.2307/2666188>
- Fahn, A. (1990). *Plant Anatomy* (4th Ed.). Pergamon Press.
- Fang, W. (2024, May). Assessment Of Agricultural Development Level Based on Hierarchical Cluster Analysis and Principal Component Analysis: Evidence from China. In *Proceedings of the 2024 9th International Conference on Mathematics and Artificial Intelligence* (Pp. 44–52). <https://doi.org/10.1145/3617731.3640069>
- Ghosh, A., Das, A., & Pandey, A. (2014). Quantitative and Qualitative Differences in Floral Characters of Plumeria Alba. *Bangladesh Journal of Botany*, 43(2), 205–212.
- Halbritter, H., & Buchner, R. (2016). Pollen Morphology of Plumeria Alba. *Grana*, 55(2), 123–134. <https://doi.org/10.1080/00173134.2015.1132648>
- Herrera-Martinez, V., Rios-Hernandez, L., Garciduenas-Pina, C., Lara-Ibarra, A., Adabache-Ortiz, A., Soria-Guerra, R. E., ... & Morales-Domínguez, J. F. (2015). Effect of Culture Conditions on Stomatal Density and Stomatal Index in Four Cactus Species. *Haseltonia*, 2015(20), 43–50. <https://doi.org/10.2985/026.020.0106>
- Joshi, J., Patel, B., & Kumar, S. (2021). Ecogeographic Divergence and Trait Variation in Gujarat Plumeria. *Indian Journal of Ecology*, 48, 312–320.
- Kobayashi, K. D., Mcconnell, J., & Griffis, J. (2019). Plumeria (Frangipani). University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources.
- Lambers, H., Chapin Iii, S. F., & Pons, T. L. (2008). *Plant Physiological Ecology*. Springer.
- Lumsden, P. J., Nicholas, J. R., & Davies, W. J. (Eds.). (2012). *Physiology, Growth and Development of Plants in Culture*. Springer Science & Business Media.
- Magurran, A. E. (2013). *Measuring Biological Diversity*. Wiley-Blackwell.

- Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., ... & Van Kleunen, M. (2010). Plant Phenotypic Plasticity in a Changing Climate. *Trends in Plant Science*, 15(12), 684–692. <https://doi.org/10.1016/j.tplants.2010.09.003>
- Onefeli, A. O., & Kehinde, L. P. (2020). Taxonomic Value of Leaf Epidermal Markers in Discriminating Some Medicinal Tree Species of Apocynaceae Juss. *Environmental Sciences Proceedings*, 3(1), 91.
- Pan, S., Liu, Z., Han, Y., Zhang, D., Zhao, X., Li, J., & Wang, K. (2024). Using The Pearson's Correlation Coefficient as The Sole Metric to Measure the Accuracy of Quantitative Trait Prediction: Is it Sufficient? *Frontiers in Plant Science*, 15, 1480463. <https://doi.org/10.3389/fpls.2024.1480463>
- Patel, P., Shah, G., & Desai, N. (2020). Diversity in Nerium Oleander: An Apocynaceae Member. *Journal of Plant Biology*, 63, 91–100.
- Piao, S., Sitch, S., Ciais, P., Friedlingstein, P., Peylin, P., Wang, T., ... & Luo, Y. (2014). Environmental Determinants of Intraspecific Variation in Plant Functional Traits. *Global Change Biology*, 20(11), 3729–3736. <https://doi.org/10.1111/gcb.12629>
- Pielou, E. C. (1969). *An Introduction to Mathematical Ecology*. Wiley.
- Prabhakar, M. (2004). Structure, Delimitation, Nomenclature and Classification of Stomata. *Acta Botanica Sinica*, 46(2), 242–252.
- Rao, R. S., & Rajput, K. S. (2012). Floral Morphology and Anatomy of Apocynaceae with Reference to Taxonomy. *Annals of Botany*, 109(1), 23–45. <https://doi.org/10.1093/aob/mcr270>
- Ridzuan, K., & Kalu, M. (2023). Comparative Micromorphology Leaf Surface of Selected Hoya Spp.(Apocynaceae) From Sarawak. *Reinwardtia*, 22(2), 69-77.
- Rr, S. (1958). A Statistical Method for Evaluating Systematic Relationships. *University of Kansas Science Bulletin*, 38, 1409–1438.
- Salim, S., Khan, M. S., & Ahmad, J. (2021). Comparative Stomatal Morphology in Apocynaceae. *Plant Biology*, 23(3), 439–450. <https://doi.org/10.1111/plb.13222>
- Shannon, C. E., & Weaver, W. (1949). *The Mathematical Theory of Communication*. University of Illinois Press.
- Sharma, M., Singh, R., & Kumar, A. (2013). Morphological and Reproductive Diversification in Allamanda Cathartica and Rauwolfia Serpentina. *Indian Journal of Horticulture*, 70(3), 380–385.
- Simpson, E. H. (1949). Measurement of Diversity. *Nature*, 163, 688. <https://doi.org/10.1038/163688>
- Singh, P., Gupta, S., & Sharma, R. (2022). Comparative Morpho-Anatomical Standardization And Chemical Profiling of Root Drugs for Distinction of Fourteen Species of Family Apocynaceae. *Botanical Studies*, 63, Article 36. <https://doi.org/10.1186/S40529-022-00342-Z>
- Su, Y. H., Liu, Y. B., & Zhang, X. S. (2011). Control Of Meristem Activity by Hormones in Plant Development. *Plant Cell Reports*, 30(9), 1563–1574. <https://doi.org/10.1007/S00299-011-1106-7>
- Sultan, S. E. (2000). Phenotypic Plasticity for Plant Development, Function and Life History. *Trends in Plant Science*, 5(12), 537–542. [https://doi.org/10.1016/S1360-1385\(00\)01770-0](https://doi.org/10.1016/S1360-1385(00)01770-0)

- Taiz, L., Zeiger, E., Moller, I. M., & Murphy, A. (2015). *Plant Physiology and Development* (6th Ed.). Sinauer Associates.
- Westerband, A. C., Funk, J. L., & Barton, K. E. (2021). Intraspecific Trait Variation in Plants: A Renewed Focus on its Role in Ecological and Evolutionary Processes. *Annals of Botany*, 127(3), 397–410. <https://doi.org/10.1093/aob/mcab006>
- Woodward, F. I., & Kelly, C. K. (1995). The Influence of CO₂ Concentration on Stomatal Density. *New Phytologist*, 131(3), 311-327.
- Yadava, R. K., Kumar, S., Singh, R., & Dubey, N. K. (2012). Genetic and Phenotypic Diversity in *Catharanthus roseus*. *Journal of Medicinal Plants Research*, 6(28), 4404–4413.
- Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S. W., Chen, H., ... & Jacobsen, S. E. (2006). Epigenetic Mechanisms Underlying Plant Development. *Cell*, 126(5), 1024–1035. <https://doi.org/10.1016/j.cell.2006.08.004>
- Zhou, Y., Xiao, S., Wang, J., Liu, J., Jiang, W., & Li, J. (2022). Floral Variation and Reproductive Success in Ornamental *Plumeria*. *Horticultural Research*, 9, 1587–1602. <https://doi.org/10.1093/hr/uhac118>
- Fang, W. (2024). Assessment of Agricultural Development Level Based on Hierarchical Cluster Analysis and Principal Component Analysis: Evidence From China. In *Proceedings of The 2024 9th International Conference On Mathematics And Artificial Intelligence* (Pp. 44-52). <https://doi.org/10.1145/3670085.3670086>
- Beentje, H. (2010). *Plant Glossary*. Royal Botanic Gardens, Kew. Richmond, Surrey.