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Exploring leaf architecture in varieties of *Hibiscus rosa-sinensis* L. (Malvaceae)

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Abstract. Leaf architecture has been successfully used in the taxonomic and systematic studies of many plant families, genera, and species but little employed at the variety level. The present study demonstrated how leaf architecture can be equally important for plant varieties as well. The leaf architecture of four *Hibiscus rosa-sinensis* L. varieties namely 'Reddy or Not', 'Nay Isa', 'Lolay', and 'Saskia De Lang' from the Institute of Plant Breeding was studied. A total of 150 leaf specimens were digitized, examined, measured, and described. The semicraspedodromous pattern of the secondary veins and the angle between the 3° to 1° vein were characters found to have taxonomic significance in describing *H. rosa-sinensis* species while the angle of the 2° vein was important for delineating *H. rosa-sinensis* varieties. The resulting dendrogram from the cluster analysis grouped all the *H. rosa-sinensis* from the outgroup and further created three subclusters that were closely related.

Keywords: leaf architecture, leaf morphology, venation patterns, *Hibiscus rosa-sinensis*, varieties.

INTRODUCTION

Leaf architecture has been recognized as a promising tool in naming and classifying plant taxa, especially in the absence of reproductive parts such as flowers and fruits (Bhat 1995; Fuller and Hickey 2005; Pacheco-Trejo et al. 2009; Laraño and Buot 2010; Lu et al. 2012; Masungsong et al. 2019; Buot 2020). The leaf provides important diagnostic features such as venation patterns, shape, and size that can help describe and establish taxonomic relationships. Through a detailed examination and documentation of leaves, even taxonomic confusion in the identity can be resolved (Baltazar and Buot 2019; Buot 2020).

Hibiscus rosa-sinensis L. (Malvaceae) is a widely cultivated ornamental plant in the tropical and subtropical regions of the world. Prized mainly for its large, showy, and colorful flowers, many breeders have successfully produced hybrids that are coming in and out of the plant market. In the Philippines, the Institute of Plant Breeding (IPB) at the University of the Philippines Los Baños has collectively released more than 40 hybrids since 1995

(Magdalita and Pimentel 2013; Magdalita and San Pascual 2021). But this is only a small part of the more than 24,000 cultivars listed in the database of the International Hibiscus Society (2023), many not officially named.

Over the years, most leaf architecture studies have focused mainly on the family, genus, and species levels. For instance, Laraño and Buot (2010) studied the leaf architecture of the Malvaceae family to present pieces of evidence of its circumscription in the Angiosperm Phylogeny Group (2003). Similarly, Bhat (1995) employed leaf architectural analysis within the genus *Hibiscus* as represented by 13 species including the most famed *Hibiscus rosa-sinensis*. We currently have little information about the significance of leaf architecture at a varietal level. Therefore, this study was conducted to fill this research gap by examining the leaf architecture of *Hibiscus rosa-sinensis* varieties.

MATERIALS AND METHODS

Four varieties of *Hibiscus rosa-sinensis* namely 'Lolay', 'Nay Isa', 'Reddy or Not', and 'Saskia De Lang' were selected for this study based on the availability of stock plants at the Institute of Plant Breeding, the University of the Philippines Los Baños. The species *Hibiscus mutabilis* L. was also included as an outgroup.

A cumulative total of 150 fresh and mature leaf samples were collected, pressed, and dried with 30 samples gathered from each kind. Digital images of the specimens were produced using MicroTek ObjectScan 1600 at the maximum resolution of 1600 dpi. Their leaf architecture was described following the manual of the Leaf Architecture Working Group (Hickey 1973; LAWG 1999; Ellis et al. 2009). ImageJ software was utilized to measure areas and angles. Data collected were analyzed using Multivariate Hierarchal Cluster Analysis in Past4. Algorithm was set to Unweighted Pair-Group Method with Arithmetic Means (UPGMA) and Euclidean distance was applied as the Similarity Index; Boot N = 10,000.

RESULTS AND DISCUSSION

The 30 collected leaf samples for each kind shared overall similar laminar features. There were only nuanced variations on the sizes and colors of the blade that were directly observed both in the fresh and dried states.

Leaves of the four *Hibiscus rosa-sinensis* varieties (Fig. 1) were generally ovate, symmetrical, serrated, and unlobed. Size can be categorized into microphyll, mesophyll, and notophyll; the base angle is either wide obtuse or obtuse; the base shape is cordate, cuneate, or convex; the apex shape is either acute or acuminate; and the apex angle can be obtuse or acute. Petiolar insertion is found at the margin (Table 1).

In terms of venation patterns, results show that the 1° vein category, 2° vein spacing, inter-2° veins, 3° vein category and course, areolation, marginal ultimate venation, and freely ending ultimate veins based on Hickey's descriptors (1973) were found similar within and among species (Table 1). Hence, these qualitative characters cannot be considered of taxonomic significance to the members of the genus and among *H. rosa-sinensis* varieties. However, it can probably have taxonomic importance when classifying higher taxa rankings.

The basal actinodromous pattern, well-developed areoles, and an incomplete marginal venation were consistent in all *Hibiscus* species as reported by Bhat (1995). The findings of the present study also indicated that these patterns remain unchanged among H. rosa-sinensis varieties. Hence, it confirms that these characters were significant in delineating the genus with other members of the family Malvaceae.

On the other hand, the category of the 2° veins in all four varieties displayed a semicraspedodromous pattern in which one of the branches terminate at the margin and the other joined the super-adjacent 2° veins. Similarly, in *Mangifera indica*, the category of the 2° vein which was camptodromous was consistent in all 30 varieties (Sharma et al. 2016). The pattern of the 2° veins can be used as a robust taxonomic criterion for delimiting species, although further validation is necessary to ascertain its consistency across varieties of other plant species.

In terms of numerical data (Table 2), *Hibiscus rosa-sinensis* had 5 primary veins and the highest vein order was identified up to 4 orders. The L: W ratio was reported in full range values for all varieties. Areole area was found smallest in *H. mutabilis* followed by 'Reddy or Not', 'Nay Isa', 'Saskia De Lang', and the largest 'Lolay'. With regards to the 2° vein angle, 'Saskia De Lang' had the largest while the outgroup species had the smallest measured angle. Lastly, the angle of the 3° veins relative to the 1° vein was found smallest at 'Lolay' followed by 'Nay Isa', 'Saskia De Lang', 'Reddy or Not', and *H. mutabilis* respectively.

Although many neglected leaf characters as a basis for taxonomic delineation due to views that these exhibit high phenotypic plasticity (Medina et al. 2016; Buot 2020), venation patterns are likely determined by genetic factors and are consistent (Roth-Nebelsick et al. 2001). Succeeding studies also vouched the findings of Roth-

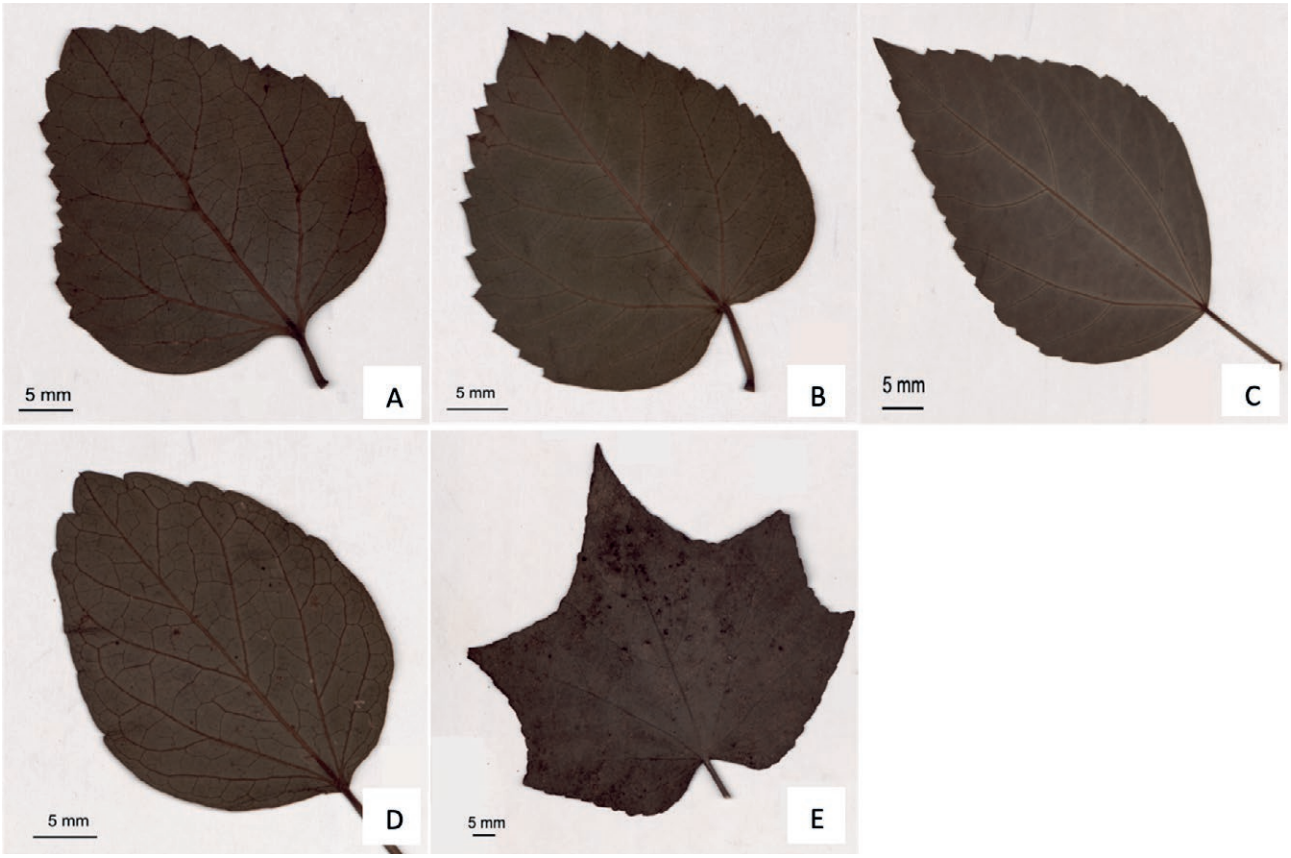

Figure 1. Photograph showing the leaf architecture details of A. *Hibiscus rosa-sinensis* 'Lolay' B. *Hibiscus rosa-sinensis* 'Nay Isa' C. *Hibiscus rosa-sinensis* 'Reddy or Not' D. *Hibiscus rosa-sinensis* 'Saskia De Lang' and E. *Hibiscus mutabilis* (outgroup).

Nebelsick et al. (2001) that leaf architecture patterns are genetically fixed (Huiet et al. 2018; Baltazar and Buot 2019; Tan and Buot 2020). In fact, accurately extracting leaf shape and venation patterns digitally for plant identification and botanical research is of interest in the field of computer vision (Cao et al. 2017).

Moreover, qualitative morphological characters of leaves cannot be solely relied upon as criteria for taxonomic and phylogenetic evaluation due to differing opinions on which characters must be considered significant (Bhat 1995; Buot 2020). It also tends to generalize characteristics that could probably have significant value when actual measurements were used in the analysis. Hence, the measured quantitative data on L: W ratio, highest vein order, no. of primary veins, 2° vein angle, the angle between the 3° and 1° vein, and areole area were the basis for the cluster analysis of this study.

The resulting dendrogram from the hierarchal cluster analysis differentiated all the *Hibiscus rosa-sinensis* species with the outgroup *Hibiscus mutabilis* at 19 Euclidean distances with bootstrap values = 100 (Fig. 2). The angle between the 3° vein in relation to the 1° vein proved to be a valuable character in depicting *H. rosa-sinensis* from the outgroup. The findings aligned with other plant genera such as *Cucumis* (Masungsong et al. 2019), *Ficus* (Loutfy et al. 2005), and *Terminalia* (Baroga and Buot 2014) where in the same character contributed to species delineation.

On the other hand, the 2° vein angle grouped the four varieties into 3 subclusters. 'Reddy or Not', a cross between *H. rosa-sinensis* and *H. schizopetalus*, separated from the IPB varieties at 9 Euclidean distances with 73 bootstrap values. The said variety was a known rootstock hybrid from Australia (International Hibiscus Society 2023). The three-way cross hybrid 'Saskia De Lang' from the IPB Diplomat Series released in honor of the Netherlands Ambassador to the Philippines (Afable 2022) formed the second subcluster with 2 other IPB varieties at 5 Euclidean distances with 100 bootstrap values. Lastly, 'Nay Isa' and 'Lolay' both from the Centennial Series were sister taxa. Therefore, the 2° angle is an important character for the taxonomical investiga-

Table 1. Qualitative leaf features of *Hibiscus rosa-sinensis* varieties.

No	Taxonomic character	*H. rosa-sinensis* 'Lolay'	*H. rosa-sinensis* 'Nay Isa'	*H. rosa-sinensis* 'Reddy or Not'	*H. rosa-sinensis* 'Saskia De Lang'	*H. mutabilis*
1	Leaf size	notophyll	notophyll	mesophyll	microphyll	mesophyll
2	Leaf shape	ovate	ovate	ovate	ovate	ovate
3	Leaf symmetry	symmetrical	symmetrical	symmetrical	symmetrical	symmetrical
4	Base angle	wide obtuse	wide obtuse	obtuse	obtuse	wide obtuse
5	Base shape	cordate	cordate	cuneate	convex	cordate
6	Apex angle	obtuse	obtuse	acute	obtuse	odd-lobed obtuse
7	Apex shape	acute	acuminate	acuminate	acute	acuminate
8	Petiole position	marginal	marginal	marginal	marginal	marginal
9	Margin Type	serrate	serrate	serrate	serrate	serrate
10	Lobation	unlobed	unlobed	unlobed	unlobed	Palmately lobed
11	1° vein category	basal actino-dromous	basal actino-dromous	basal actino-dromous	basal actino-dromous	basal actino-dromous
12	2° vein category	semicraspe-dodromous	semicraspe-dodromous	semicraspe-dodromous	semicraspe-dodromous	craspe-dodromous
13	2° vein spacing	increasing to base	increasing to base	increasing to base	increasing to base	increasing to base
14	Inter-2° veins	strong	strong	strong	strong	strong
15	3° vein category	alternate percurrent	alternate percurrent	alternate percurrent	alternate percurrent	alternate percurrent
16	3° vein course	sinuous	sinuous	sinuous	sinuous	sinuous
17	Areolation	well-developed	well-developed	well-developed	well-developed	well-developed
18	Marginal ultimate venation	incomplete; toothed	incomplete; toothed	incomplete; toothed	incomplete; toothed	incomplete; toothed
19	Free-ending veins	absent	absent	absent	absent	absent

Table 2. Numerical data on venation patterns of *Hibiscus rosa-sinensis* varieties.

Name of Taxa	L: W Ratio	Highest Vein Order	No. of 1° vein	2° vein angle	Angle between 3° to 1° veins	Areole Area (mm^2)
H. rosa-sinensis 'Lolay'	1:1	4	5	51.75	37.01	8.55
H. rosa-sinensis 'Nay Isa'	7:6	4	5	52.04	38.39	5.45
H. rosa-sinensis 'Reddy or Not'	2:1	4	5	57.22	41.67	2.67
H. rosa-sinensis 'Saskia De Lang'	13:9	4	5	46.98	39.67	7.33
H. mutabilis	11:12	5	7	64.00	53.00	1.28

tions of *H. rosa-sinensis* varieties. Leaf characters such as highest vein order, number of primary veins, areole area, and L: W ratio were deemed insignificant.

CONCLUSIONS

This paper demonstrated the significance of leaf architecture in delineating different *H. rosa-sinensis* varieties namely 'Lolay', 'Nay Isa', 'Saskia De Lang', and 'Reddy or Not'. The findings confirmed that leaf architecture is a tool helpful not just in higher taxa but also in classifying varieties, especially in the absence of reproductive structures. The 2° vein angle was an important taxonomic character for the delineation of *Hibiscus rosa-sinensis* varieties while the 2° vein category and the 3° vein angle in relation to the 1° vein were both important for the species. It is suggested to further explore leaf architecture in varieties of other plant species.

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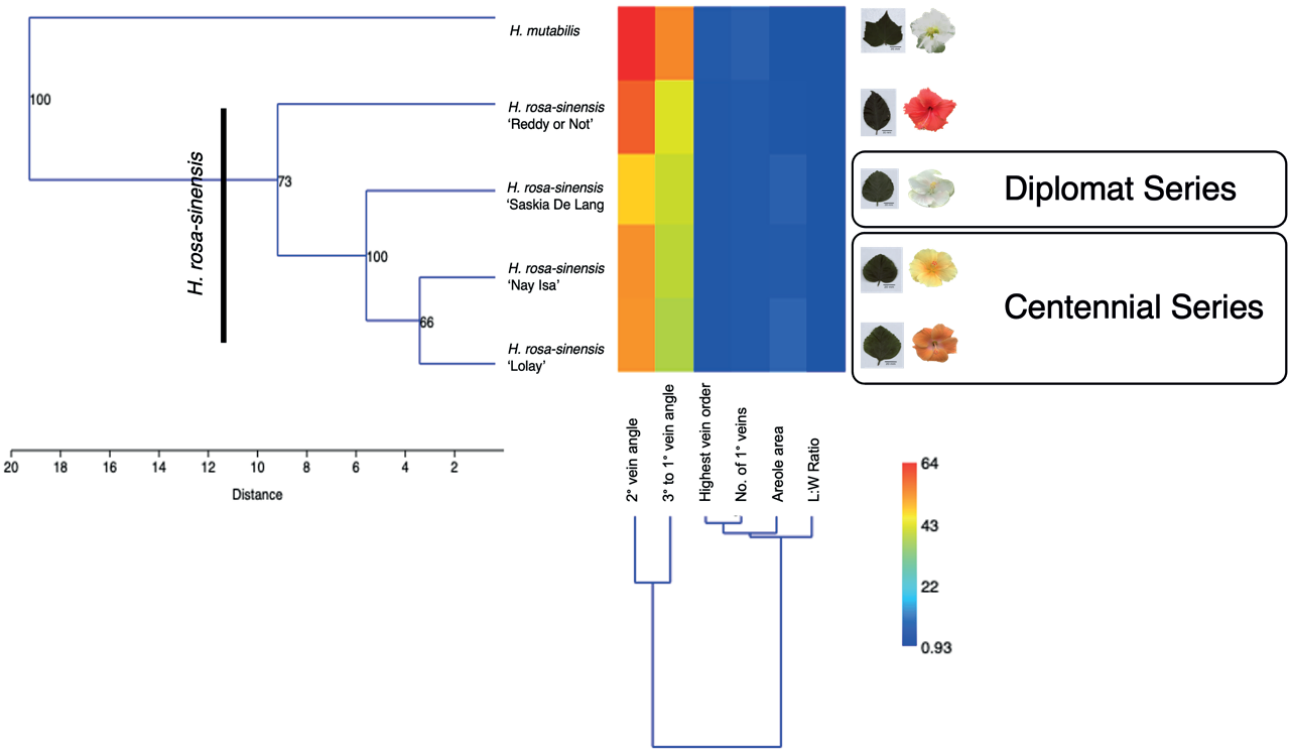

Figure 2. Dendrogram showing clustering of *Hibiscus rosa-sinensis* varieties using Unweighted Pair-Group Method with Arithmetic Means (UPGMA) with Euclidean Distance as the Similarity Index; Bootstrap values = 10,000.

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