Applications of leaf architecture characters in delineating selected species and infraspecific taxa of Genus Cucumis L. (Cucurbitaceae)

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Abstract. Leaf venation characters of twelve (12) Cucumis taxa, comprised of eight species, three subspecies, and three varieties were examined to determine patterns of leaf architectural characters and delineate these accessions at the species, subspecies, and variety level. The leaf architecture of twenty-seven accessions representing six taxa were examined and described in this study. Additionally, data on the leaf architecture of another six taxa (represented by 57 accessions) published by Masungsong et al. (2022) were incorporated in the analysis. Results revealed that the twelve (12) taxa of Cucumis primarily varied in blade class, apex angle, primary vein size, secondary vein spacing, tertiary vein angle to primary vein, and areole development. These variations were found consistent with Cucumis species examined in previous studies. A dichotomous key was constructed for the twelve Cucumis taxa using leaf characters derived. Cluster analysis (based on UPGMA) formed two major clusters at 0.130 Gower distance. Cluster I includes Cucumis melo subsp. melo, C. pustulatus, C. melo var. flexuosus, C. melo subsp. agrestis, and C. melo var. texanus which exhibited secondary vein spacing that increases towards the base, while Cluster II is composed of the remaining Cucumis taxa, all displaying irregular secondary vein spacing. Furthermore, subclusters were formed based on tertiary vein angle in relation to primary vein, blade class, apex angle, and primary vein size. Results showed that utilizing leaf architecture is an effective technique to describe, characterize and delineate closely related taxa with many similar characters.

Keywords: Cucumis, Cucurbitaceae, leaf architecture, leaf venation, species delineation.

INTRODUCTION

Plant reproductive structures (flowers and fruits) are the most common and widely used taxonomic features for the identification and classification of plant species. However, these structures can be challenging to investigate due to their seasonality (da Silva et al. 2015). The use of plant vegetative charac-
ters such as the leaves are similarly important since they are present throughout the plant’s life span (Buot 2020; Masungsong et al. 2019a). Leaf architecture is one of the most valuable tools that is being utilized in many recent taxonomic studies (Buot 2020). It is very significant in describing, classifying, and identifying taxa with confusing characters regardless of its often-debated high phenotypic plasticity. Although some leaf features like shape and size may be plastic, venation patterns are genetically fixed according to Roth-Nebelsick et al. (2001) and Huiet et al. (2018).

Leaf architectural characters have been proven useful in delineating species of Philippine Cinnamomum Schaeff. (Celadiña et al. 2012), Camellia L. (Lu H et al., 2012), Terminalia L. (Baroga 2014), Saxifraga Tourn. ex L. (Zhang 2015), Bauhinia Plum. ex L. (Lin et al. 2015), Diplazium Sw. (Conda et al., 2017), Adiantum L. (Huiet et al., 2018), Dioscorea Plum. ex L. (Antonio and Buot 2021), and Hoya R. Br. (Salvaha and Buot 2014; Villareal and Buot 2015; Jumawan and Buot 2016; Torrefiel and Buot, 2017; Tan and Buot 2018; Baltazar and Buot, 2019; Paguntalan and Buot 2019). These researches revealed that leaf architecture has enormous potential as a tool for solving taxonomic problems.

The genus Cucumis comprises a variety of cultivated species classified as food crops. Cucumis sativus and C. melo are just two of the many economically important members of the genus. Several leaf architectural studies on Cucumis have been conducted and contributed to the identification, classification, and delineation of the genus (Rao and Rao 2015; Masungsong et al. 2019a; Masungsong et al. 2019b; Averion-Masungsong and Buot 2020; Masungsong et al. 2022).

Nevertheless, a significant number of Cucumis species remain taxonomically problematic in terms of identification and classification due to overlapping general morphology. Currently, there are seventeen (17) Cucumis species that are conserved in the Hortanova Farm and Research Center (HFRC) gene bank of Eastwest Seed Company Inc. Identification of these species has increasingly become a challenge when reproductive structures are absent.

This study assessed the diversity of leaf architectural characters from various accessions of twelve (12) Cucumis taxa, including eight (8) species, three (3) subspecies, and three (3) varieties. It analyzed the leaf architecture of six (6) taxa that were not included in the previous Cucumis leaf architecture studies of Masungsong et al. (2019a), Masungsong et al. (2019b), Averion-Masungsong and Buot (2020); and Masungsong et al. (2022). By combining the current research results with that of previous studies, the accessions were classified and the species, subspecies, and varieties of Cucumis were delineated based on leaf architecture.

MATERIALS AND METHODS

Leaf sample collection

Twenty-seven (27) accessions representing six (6) Cucumis taxa with previously unexamined leaf architecture (Table 1), were systematically planted at the HRFC, East West Seed Company Inc. screen house, in Lipa City, Batangas Philippines. Three (3) leaves from three (3) standing crops, were collected from each accession. A total of two hundred forty-three (243) leaves were collected, pressed, dried, and observed under a dissecting microscope. Herbarium specimens were prepared from each accession, then deposited at the Plant Biology Division Herbarium (PBDH), Institute of Biological Sciences, University of the Philippines, Los Baños.

Secondary data

In the work of Masungsong et.al. (2022), fifty-seven (57) accessions representing six (6) taxa of Cucumis were examined. The leaf architecture descriptions of C. melo subsp. agrestis (Naudin) Pangalo, C. melo var. flexuosus (L.) Naudin, C. melo var. texanus Naudin, C. sativus L., C. sativus var. hardwickii (Royle) Gabaev, and C. zambianus Widrl, J.H.Kirbr., Ghebret. & K.R.Reitsma were reported and therefore will not be elaborated on in this paper. Nevertheless, the data on these six taxa will be incorporated in the analysis to provide a more comprehensive coverage of the genus Cucumis.

Leaf venation analysis

Twelve (12) general leaf- and eleven (11) venation characters used in previous Cucumis studies were also observed and measured in this study. This is to ensure consistent classification for all the Cucumis taxa and accessions used. The leaf venation characters were based from Hickey’s (1973), LAWG’s (1999), and Elli’s et al. (2009) leaf architectural descriptors. Leaf architecture data generated from this study were combined with data from the study of Masungsong et al. (2022) to construct a dichotomous key to twelve Cucumis taxa. The combined data was also used to perform cluster analysis based on the Unweighted Pair Group Method using Averages (UPGMA) and Gower distance correlation, using the PAleontological Statistics (4.04) software.
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**RESULT AND DISCUSSIONS**

**General leaf characters and venation characters of *Cucumis* species**

Based on the data gathered from a total of eighty-four (84) accessions (27 from this study and 57 from the study of Masungsong et al. (2022), the twelve (12) *Cucumis* taxa six (6) from this study and six (6) from Masungsong et al. (2022) share many common leaf architecture traits (Table 2 and Masungsong et al. (2022). In fact, all twelve taxa displayed the same traits in nine (9) out of the twelve (12) general leaf characters and eleven (7) out of the eleven (11) leaf venation characters.

The unifying features of the leaves of these *Cucumis* taxa are: simple leaf organization, orbiculate and symmetrical lamina, convex apex, lobate base with wide obtuse angle, serrated leaf margins, spinose tooth apex, palmate lobation, actinodromous (i.e., arising from the same point of origin) suprabasal primary veins that are straight-branched, craspedodromous (i.e., pinnately arranged and terminating at the leaf margins) secondary veins with two-pair acute basal secondaries, alternate percurrent tertiary veins, regular, polygonal and reticulate quaternary veins, and looped marginal ultimate venation. These shared features can be used to distinguish the leaves of *Cucumis* species from other genera.

Variations of leaf characters were observed in their blade class and apex angle. Among the 12 taxa, only *C. ficifolius* exhibited a microphyll to notophyll blade class while other species have mesophyll to macrophyll blade class (*C. zambianus, C. melo var. flexuosus, C. sativus var. hardwickii, and C. melo subsp. agrestis*), mesophyll blade class (*C. sativus, C. pustulatus, C. melo subsp. melo* and *C. melo var. texanus*) and strictly notophyll blade class (*C. dipsaceus, C. africanus, and C. myriocarpus subsp. myriocarpus*). Blade class has been used to delineate species, infraspecific taxa, and accessions. Similar to the study of Masungsong et al. (2019b) it differentiated *C. anguria* from *C. anguria var. longaculeatus*. However,
two accessions of *C. anguria* displayed a different blade class clustering them with *C. anguria* var. *longaculeatus* implying the close relationship of the two aforementioned varieties. Some leaf architecture studies were also able to delineate species using blade class (Baroga and Buot 2014; Kpadehyea and Buot 2014; Tan and Buot 2018; Antonio and Buot 2021). Moreover, blade class was used as an identification tool for *Glycine max* (L.) Merr. accessions (Chen and Nelson 2004). However, in this study blade class alone is not enough to distinguish species as we observed different types of blade class in *C. melo* subspecies and varieties, as well as in *C. sativus* varieties. All the *Cucumis* taxa exhibited odd-lobed and obtuse apex angles with the exception of *C. melo* var. *flexuosus* (obtuse apex angle), *C. melo* var. *texanus* (obtuse to odd-lobed obtuse apex angle), and *C. sativus* and *C. sativus* var. *hardwickii* (odd-lobed acute apex angle). Similar with the result of this study, diversity of apex angle is also observed by Masungsong et al. (2019a, 2019b) in other *Cucumis* species. The apex angle of odd-
lobed leaves can represent the degree of lobation in *Cucumis* (Nandyal et al. 2013).

Variations were observed in their primary vein size, secondary vein spacing, tertiary vein angle in relation to primary vein category, and areolation. In terms of primary vein size, the *Cucumis* accessions can be grouped into four: (1) those having weak primary vein size (*C. melo* subsp. *agrestis*, *C. melo* var. *texanus*, *C. melo* var. *flexuosus*, *C. zambianus*, *C. sativus*, *C. sativus* var. *hardwickii*, *C. pustulatus*, and *C. melo* ssp. *melo*), (2) moderate primary vein size (*C. dipsacus* and *C. africanus*), (3) weak to moderate primary vein size (*C. ficifolius*), and (4) weak to stout primary vein size (*C. myriocarpus* subsp. *myriocarpus*). Hence, primary vein size can be used to delineate *C. ficifolius* and *C. myriocarpus* subsp. *myriocarpus* from the rest of the taxa.

Studies have shown that primary vein size and other primary vein characteristics can be used in classifying species, as demonstrated in *Podocarpus* L. Her. ex Pers. (Salvaña et al. 2018) and *Anthurium* Schott subsections (Mantovani et al. 2009). Two groups were formed on the basis of secondary vein spacing, *Cucumis pustulatus* and all the subspecies and varieties of *C. melo* have increasing spacing towards the base secondary vein while other species have irregular secondary vein spacing. These two character states were also observed in other *Cucumis* species (Masungsong et al. 2019b). Just like apex angle, secondary vein spacing is associated to lobation. The extent of lobation is inversely proportional to leaf surface area. Lobation is important in discerning specific vein patterns in the leaf and it can also reflect evolutionary relationships between taxa (Yang et al. 2022).

Most of the *Cucumis* taxa display obtuse tertiary vein angle to primary vein while *C. melo* subsp. *agrestis*, *C. melo* subsp. *texanus*, *C. melo* subsp. *flexuosus* and *C. zambianus* have an acute tertiary vein angle to primary vein. This character delineated *C. metuliferus* from *C. myriocarpus* as observed in the study of Masungsong et al. (2019a, 2019b). Nelson and Dengler (1997) also indicated that intra- and interspecific variations can be seen in higher vein orders like tertiary veins. For the areole development, *C. zambianus* accessions can be distinguished as they have moderately developed areolation while the other taxa have well-developed areolation. Areolation was also cited by Kpadehyea and Buot (2014) as one of the most useful leaf characters in differentiating species and infraspecific taxa of *Mussaenda* Burm. ex L. in the Philippines.

A dichotomous key was produced, using the leaf architecture descriptions of the examined taxa. The usefulness of leaf architecture, especially venation characters in delineating and describing *Cucumis* species is apparent. Similar findings were reported by Celadiña et al. (2012) in Philippine *Cinnamomum* species, Conda et al. (2017) in selected *Diplazium* species, Salvaña and Buot (2014) in *Hoya* species, and Pulan and Buot (2014) in Philippine *Shorea* Roxb. ex C.F. Gaertn. species.

### Key to twelve taxa of *Cucumis* L. based on leaf architecture

<table>
<thead>
<tr>
<th>Description</th>
<th>Taxon</th>
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<tbody>
<tr>
<td>2° vein craspedodromous with irregular spacing</td>
<td>...............</td>
</tr>
<tr>
<td>Convex apex shape, odd lobed obtuse apex angle</td>
<td>...............</td>
</tr>
<tr>
<td>Convex apex shape, odd lobed acute apex angle</td>
<td>...............</td>
</tr>
<tr>
<td>3° vein alternate percurrent with obtuse 3° vein angle to 1°</td>
<td>...............</td>
</tr>
<tr>
<td>3° vein alternate percurrent with acute 3° vein angle to 1°</td>
<td>...............</td>
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<tr>
<td>1° vein actinodromous suprabasal, with moderate vein size</td>
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<tr>
<td>1° vein actinodromous suprabasal, with weak to moderate or weak to stout vein size</td>
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<tr>
<td>Palmately lobed, notophyll with 0.97 to 1.16 L:W ratio</td>
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<tr>
<td>Palmately lobed, notophyll with 0.97 to 1.60 L:W ratio</td>
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<tr>
<td>Weak to moderate 1° vein size</td>
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<tr>
<td>Weak to stout 1° vein size</td>
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<tr>
<td>Well-developed areolation, macrophyll with 0.95 to 1.17 L:W ratio</td>
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<tr>
<td>Well-developed areolation, macrophyll with 1.03 to 1.12 L:W ratio</td>
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<tr>
<td>2° vein craspedodromous with increasing towards the base spacing</td>
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<tr>
<td>Convex apex shape with odd lobed obtuse apex angle</td>
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<td>Convex apex shape with obtuse apex angle</td>
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<td>3° vein alternate percurrent with obtuse 3° vein angle to 1°</td>
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<td>3° vein alternate percurrent with acute 3° vein angle to 1°</td>
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<tr>
<td>4° vein regular polygonal reticulate, mesophyll with 0.97 to 1.03 L:W ratio</td>
<td>...............</td>
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<tr>
<td>4° vein regular polygonal reticulate, mesophyll with 0.99 to 1.06 L:W ratio</td>
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Species and infraspecific delineation of Cucumis based on leaf architecture

Cluster analysis classified the eighty-four (84) accessions into two major clusters. The UPGMA dendrogram with a cophenetic correlation of 0.9031 and Gower distance of 0.130 is shown in Figure 1. Cluster I includes all accessions of \( C. \) melo var. flexuosus, \( C. \) melo var. texanus, \( C. \) pustulatus, \( C. \) melo subsp. melo and \( C. \) melo subsp. agrestis characterized by secondary vein spacing increasing towards the base. On the other hand, Cluster II is composed of all accessions of \( C. \) sativus, \( C. \) sativus var. hardwickii, \( C. \) africanus, \( C. \) ficifolius, \( C. \) myriocarpus subsp. myriocarpus and \( C. \) zambianus with irregular secondary vein spacing. This result is congruent with the results of the previous study (Masungsong et al. 2019a 2019b) wherein all the studied \( C. \) cucumis species and accessions were primarily delineated on the basis of secondary vein spacing. Thus, secondary vein spacing can be used as a delineating character of \( C. \) cucumis species and infraspecific taxa.

The regularity in the spacing of leaf veins is a remarkable trait, especially in recently evolved monocots and dicots wherein reticulate venation is apparent despite the differences in leaf shape. On account of its regularity, it is a stable character that can be used in distinguishing taxonomic groups. There are observed differences in the uniformity of the veins which can either be primary, secondary, tertiary, or higher vein orders (Nelson and Dengler 1997). The spacing indicates the strength of the support on the blade. Leaves with palmate lobation need shorter spacing of secondary veins to support the lobed part since it is relatively distant from the midvein and petiole. Increasing spacing of secondary veins towards the base implies that the petiole provides support to the blade.

In the first cluster, \( C. \) melo subsp. melo and \( C. \) pustulatus formed a separate sub-cluster due to the similarity in tertiary vein angle in relation to primary vein which is obtuse. \( C. \) cucumis melo var. flexuosus (accessions 601 and 603) and \( C. \) melo subsp. agrestis (accessions 510, 503, 466, and 487) formed another sub-cluster as blade class for these accessions were similar (macrophyll blade class). Blade class is mainly based on the leaf surface area, with macrophyll leaves having a larger surface area than microphyll leaves. Blade classes can be used as a tool in identifying the different accessions of \( C. \) cucumis. This character has been proven to be useful in the classification of \( Glycine \) max accessions (Chen and Nelson 2004). The set of leaf characters used in this study was able to separate all the subspecific taxa of \( C. \) melo from the rest of the species, except for \( C. \) pustulatus. The subclusters correspond to the different subspecies and varieties of \( C. \) melo. As seen in Figure 1, accessions of \( C. \) melo subsp. melo and \( C. \) pustulatus are very similar to each other. It might be worth re-examining the two accessions of \( C. \) pustulatus to check for possible misclassification or mislabeling of the plant materials used.

In the second cluster, \( C. \) zambianus accessions formed a separate sub-cluster for having an acute tertiary vein angle in relation to primary vein and apex angle separates accessions of this species into two. \( C. \) cucumis myriocarpus subsp. myriocarpus accessions 412 and 414 were separated from the other accessions of the species for having an acute tertiary vein angle in relation to primary vein. Accessions of \( C. \) africanus (250, 253, 518, 525, 526), \( C. \) dipsaceus (446, 576, 577, 578, 579), \( C. \) ficifolius (488, 257, 258, 259), \( C. \) myriocarpus subsp. myriocarpus (411, 413, 415) formed a separate sub-cluster due to similarity in apex angle (odd lobed obtuse). Sub-cluster formed by \( C. \) sativus and \( C. \) sativus var. hardwickii accessions was supported by similarity in primary vein size which is weak. While clustering of accessions from the same species was clearly observed in \( C. \) zambianus and \( C. \) sativus (with the two varieties joining in one subcluster), \( C. \) africanus and \( C. \) dipsaceus appear to have very similar leaf character traits. Some accessions of different species were observed to form a group with other species. \( C. \) cucumis myriocarpus subsp. myriocarpus accessions 411, 413, and 415 were separated from accessions 412 and 414 due to tertiary vein angle in relation to primary vein. This implies that, within the same species, the tertiary vein angle in relation to primary vein may differ which can cause a range of character states general to all accession of the species. It can be indicated, based on the result that \( C. \) myriocarpus subsp. myriocarpus accessions have acute to obtuse tertiary vein angle in relation to primary vein. In terms of blade class, \( C. \) myriocarpus subsp. myriocarpus accession 411 also separated from other accessions of the species. Similar to tertiary vein angle in relation to primary vein, it can also be indicated that \( C. \) myriocarpus subsp. myriocarpus accessions have a range of blade classes from notophyll to mesophyll. Similarly, \( C. \) ficifolius accession 488 and 258 separated from accession 257 and 259 for exhibiting notophyll blade class and weak primary vein size. This result also implies that there is a range of character states for the aforementioned leaf.
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As evidenced in the dendrogram, this cluster analysis results where some accessions of *Cucumis* species grouped with other accessions of other *Cucumis* species were attributed to their leaf characters’ similarities and differences therefore can be considered as a range of character states for the different *Cucumis* species and accessions. Nevertheless, similarities of leaf architectural characters of this

**Figure 1.** Unweighted Pair Group Method using Averages (UPGMA) dendrogram based on Gower distance (0.130), of eighty-four accessions representing twelve taxa of *Cucumis*: two major clusters formed based on secondary vein spacing; Cluster I: *Cucumis* species accessions with increasing spacing towards the base secondary vein (●) and Cluster II: *Cucumis* species accessions with irregular spacing secondary vein (□).
species support assigning these accessions under the same species.

The distinct leaf characters obtained, the dichotomous key constructed, and the results of the cluster analysis for the 12 Cucumis taxa examined proved the reliability of leaf architecture as a tool in identifying and classifying species within the genus and at a certain level, subspecies and varieties within the same species.

CONCLUSION

The Cucumis accessions used in this study can be classified at the species- and infra-specific levels using leaf architecture, especially venation patterns. This approach can be successfully used to identify Cucumis species and accessions, as well as other morphologically similar species. In order to solve the issue of resource and space conservation, it can also act as a guide for gene banks, seed firms, and repositories where various accessions of species are preserved thus, aids in planning and management.

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