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Original Article

Leaf Architectural Variations among Species and Accessions of Genus *Cucumis* L.

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ABSTRACT: Taxonomic identification of *Cucumis* species and accessions in the gene bank are challenging among researchers due to morphological similarities when reproductive parts are not available. Variations in leaf architectural characters are commonly used to illustrate relationships among interspecific and intraspecific taxa. In this study, leaf architectural characters were used to determine variations among selected species and accessions of *Cucumis*. Five species of *Cucumis* were used, namely: *C. melo*, *C. myriocarpus*, *C. anguria*, *C. metuliferus*, and *C. anguria* var. *longaculeatus*. Ten accessions per species were examined, measured and described. Leaf architectural characterization was based on the established categories of Hickey (1973), LAWG (1999) and Ellis *et al*. (2009). Leaf architectural characters were subjected to cluster analysis. Similarities among *Cucumis* species and accessions examined include simple leaf, elliptic with distinct serrations at the margins, actinodromous suprabasal primary vein, craspedodromous secondary vein, regular polygonal reticulate quaternary vein and looped marginal ultimate venation. Results also showed that variations in leaf architecture among *Cucumis* species and accessions can be observed in secondary vein spacing, laminar shape, blade class, apex angle and primary vein size. These variations were supported with the result of cluster analysis wherein *Cucumis* species and accessions were successfully delineated based on these characters. Based on the results, leaf architecture is a useful tool in describing, classifying and delineating species and accessions of *Cucumis*.

INTRODUCTION

Leaf architecture nowadays plays a vital role in the identification and classification of both interspecific and intraspecific taxa because of its proven usefulness in the field of plant taxonomy. This argument is due to the fact that most of the plants collected or preserved, lack reproductive structures and it is difficult to identify species using vegetative parts due to morphological resemblance. Hickey (1973) defined leaf architecture as the placement of form of elements constituting the outward expression of leaf structure, including venation pattern, marginal configuration, leaf shape, and gland position. So even though there are issues concerning leaf plasticity, leaf venation is said to be very

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unique and enormously diverse within and across species since its design and function have a very important role in plant performance (Roth-Nebelsick *et al*., 2001). Moreover, variations of these characters have been very useful in distinguishing and delineating taxa. Several controversial species had been described and distinguished using leaf architectural characters (*Psychotria* spp., *Psidium* spp., *Hoya* spp., *Diplazium* spp., *Shorea* spp., *Cinnamomum* spp.).

Genus *Cucumis* is one of the most studied taxa in Cucurbitaceae because of its economic importance. Among *Cucumis* species, *C. melo* is the mainly cultivated species due to its disease resistance characteristic. Several studies have been conducted on the classification of the genus but most of them were based on its molecular phylogenetics (Renner and Schaefer, 2008; Telford *et al*., 2011; Rasool *et al.*, 2016); morphological descriptions and characterization of reproductive structures (De Wilde and Duyfjes, 2007; Ajuru and Okoli, 2013); and leaf epidermal structures (Jibril and Jakada, 2015). However, studies on leaf architectural characters that can be very useful when it comes to identifying and classifying the accessions have never been carried out. Aside from resolving problematic species, leaf architecture can also be used in describing and delineating various accessions of a particular species. Through this, enhancing the utilities and applications of leaf architecture in characterizing intraspecific taxa becomes important. In some repositories, several accessions are almost unidentifiable without the presence of reproductive parts. In Hortanova Farm and Research Center, Eastwest Seed company, Inc., for example, it became a challenge to identify numerous accessions of *Cucumis* species since some of these exhibit similar leaf morphology.

This study was the first attempt to examine leaf architectural variations among species and accessions of *Cucumis*. Researchers focusing on *Cucumis* species accessions (Cucurbitaceae) encounter difficulties in taxonomic identification due to apparent morphological similarities brought about by inherent plasticity of characters when they are subjected to diverse environmental conditions. Early and recent studies failed in separating the cultivated accessions. This study provided good insights not only on the use of leaf architecture but also proper identification of accessions which will definitely be of great help in breeding programs for *Cucumis*.

MATERIALS AND METHODS

Leaf samples from five systematically planted *Cucumis* species were examined in this study namely: *C. anguria*, *C. anguria* var. *longaculeatus*, *C. melo*, *C. metuliferus* and *C. myriocarpus* (Table 1). Three leaves were collected from three standing plants of each of the ten accessions of the five species at the Hortanova Farm and Research Center, East-west Seed Inc., Lipa City, Batangas, Philippines.

A total of four hundred fifty collected leaf samples were pressed, dried, and examined under a dissecting microscope. The leaf architecture pattern of the leaf samples was described based on Hickey's (1973), LAWG's (1999) and Ellis' *et al*. (2009) leaf architectural characteristics and descriptors. Measurements were done using a ruler, a caliper, and a protractor. Eleven (11) general leaf and vein characters were used in examining the collected leaf samples. Leaf characters include: (1) blade class, (2) laminar shape, (3) laminar symmetry, (4) length-width ratio, (5) apex shape, (6) apex angle, (7) base shape, (8) base angle, (9) margin, (10) tooth apex and (11) lobation. Vein characters include primary vein (category, size, course), secondary vein (category,

Table 1. Details of standing *Cucumis* species and accessions used in the study.

SCIENTIFIC NAMES	PLACE OF ORIGINS	ACCESSION NUMBERS
Cucumis melo	Senegal	GB-000367
	Afghanistan	GB-000331
	Mandalay	GB-000330
	Zambia	GB-000389
	Ecuador	GB-000359
	No data	GB-000318
	No data	GB-000366
	India	GB-000113
	China	GB-000263
	Dominican Republic	GB-000338
Cucumis myriocarpus	South Africa	GB-000407
	No data	GB-000635
	South Africa	GB-000411
	South Africa	GB-000409
	Zimbabwe	GB-000413
	Australia	GB-000404
	South Africa	GB-000408
	Zimbabwe	GB-000414
	South Africa	GB-000412
	South Africa	GB-000406
Cucumis anguria	Mexico	GB-000545
	Zimbabwe	GB-000550
	Iran	GB-000532
	Zimbabwe	GB-000547
	Zimbabwe	GB-000548
	No data	GB-000062
	Zimbabwe	GB-000546
	Zimbabwe	GB-000530
	Ethiopia	GB-000531
	Zimbabwe	GB-000554
Cucumis metuliferus	Zimbabwe	GB-000624
	Zimbabwe	GB-000627
	Zambia	GB-000632
	Zambia	GB-000631
	South Africa	GB-000618
	Soviet Union, former	GB-000621

Table 1. (Continued).

SCIENTIFIC NAMES	PLACE OF ORIGINS	ACCESSION NUMBERS
	Netherlands	GB-000620
	South Africa	GB-000619
	Netherlands	GB-000622
	Zimbabwe	GB-000625
Cucumis anguria var *longaculeatus*	South Africa	GB-000560
	Zambia	GB-000568
	South Africa	GB-000559
	South Africa	GB-000558
	Zambia	GB-000567
	Namibia	GB-000565
	Zambia	GB-000570
	Zambia	GB-000569
	Brazil	GB-000562
	Zimbabwe	GB-000571

spacing, angle), tertiary vein (category, angle in relation to primary vein), quaternary vein category, marginal ultimate venation and areole development. Cluster analysis was done using PAleontological STatistics (PAST) version 3.14 (Hammer *et al*., 2001) and a dendrogram was constructed using Euclidean distance, two-way analysis and Unweighted Pair-Group Method using Averages (UPGMA) as linkage method.

RESULTS

All examined species and accessions of *Cucumis* shared similar leaf characters in terms of leaf type, shape, margin, toothed apex. These and other general leaf characters of *Cucumis* are shown in Table 2. In terms of venation patterns, *Cucumis* species and accessions had craspedodromous 2° veins, alternate percurrent 3° veins and regular polygonal reticulate 4° veins. Suprabasal 1° veins were actinodromous and marginal ultimate venation was looped. Variations in leaf characters were observed in apex angle, blade class, lobation, vein size, vein spacing, vein angle and areole development.

Table 2. General Leaf Characters of the *Cucumis* leaves.

LEAF MORPHOLOGY	CHARACTERS
Leaf organization	Simple
Lamina shape	Elliptic (sub-orbiculate to orbiculate)
Symmetry	Symmetrical
Apex	

Table 2. (Continued).

LEAF MORPHOLOGY	CHARACTERS
Shape	Convex
Angle	Mostly odd-lobed obtuse, some obtuse and odd-lobed acute
Base	
Shape	Lobate
Angle	Wide obtuse
Margin	Serrate
Tooth apex	Spinose
Blade class	Notophyll to mesophyll
Lobation	Mostly palmately lobed, some unlobed
Venation pattern	
1° vein	
Category	Actinodromous suprabasal
Size	Weak to moderate
Course	Straight branched
2° vein	
Category	Craspedodromous
Spacing	Mostly irregular spacing, some increasing towards the base
Angle	Two-pair acute basal secondaries
3° vein	
Category	Mostly alternate percurrent, some random reticulate
Angle to 1°	Mostly obtuse, some acute
4° vein	
Category	Regular polygonal reticulate
Marginal ultimate venation	Looped
Areole development	Mostly moderately developed some well -developed,

Table 3 shows the general character description and unifying leaf architectural characters of *Cucumis* species and accessions. All *C. melo* accessions exhibited secondary vein spacing which was increasing towards the base separating these accessions from other species and accessions of *Cucumis*. Variations in primary vein size distinguished the accessions of *C. melo.* Nine accessions (GB-000331, GB-000330, GB-000389, GB-000366, GB-000113, GB-000359, GB-000263, GB-000338, Ctl1 0062) had weak primary vein size and three accessions (GB-000367, GB-000318, Ctl2 (Diosa)), had moderate primary vein size. On the other hand, all accessions of four other species

exhibited irregular vein spacing. Accessions of these species were distinguished based on the variations in other leaf architectural characters. In *C. metuliferus*, accessions primarily differed in apex angle. Six accessions (GB-000624, GB-000627, GB-000618, GB-000619, GB-000632, GB-000631) had odd-lobed acute apex angle while four accessions (GB-000625, GB-000620, GB-000621, GB-000622) had odd-lobed obtuse apex angle. All accessions of *C. anguria* var *longaculeatus* had mesophyll blade class. Surprisingly, two accessions of *C. anguria* namely GB-000062 and GB-000554, also had mesophyll leaf which separates them from other accessions of the species. Based on this leaf architectural character, it could be suggested that these two accessions belong to *C. anguria* var *longaculeatus*. Other accessions of *C. anguria* had notophyll blade class. Furthermore, one accession of *C. myriocarpus* (GB-000406) had sub-orbiculate laminar shape which differs from other accessions (GB-000406, GB-000411, GB-000409, GB-000404, GB-000408, GB-000635, GB-000413, GB-000414, GB-000412) with orbiculate laminar shape.

Table 3. General and unifying leaf architecture characters of five *Cucumis* species.

SPECIES	GENERAL CHARACTERS	UNIFYING LEAF ARCHITECTURE CHARACTERS
Cucumis melo	**Lamina** orbiculate with convex apex, obtuse apex angle, lobate base, wide-obtuse base angle and spinose tooth apex, symmetrical, mesophyll, serrated, unlobed or palmately lobed. **Venation** actinodromous suprabasal, straight branched, weak to moderate **1°; 2°** craspedodromous with increasing towards the base spacing and two pair acute basal secondaries angle; **3°** alternate percurrent and angle with respect to 1° obtuse; **4°** regular polygonal reticulate; **Marginal ultimate venation** looped; **Areoles** well-developed.	Secondary vein spacing increasing towards the base.
Cucumis myriocarpus	**Lamina** sub-orbiculate to orbiculate with convex apex, odd-lobed obtuse apex angle, lobate base, wide-obtuse base angle and spinose tooth apex, symmetrical, notophyll, serrated, palmately lobed. **Venation** actinodromous suprabasal, straight branched, moderate **1°; 2°** craspedodromous with irregular spacing and two pair acute basal secondaries angle; **3°** random reticulate and angle with respect to **1°** obtuse; **4°** regular polygonal reticulate; **Marginal ultimate venation**	3° vein category random reticulate

Table 3. (Continued).

SPECIES	GENERAL CHARACTERS	UNIFYING LEAF ARCHITECTURE CHARACTERS
	looped; **Areoles** moderately to well-developed.	
Cucumis anguria	**Lamina** orbiculate with convex apex, odd-lobed obtuse apex angle, lobate base, wide-obtuse base angle and spinose tooth apex, symmetrical, notophyll, serrated, palmately lobed. **Venation** actinodromous suprabasal, straight branched, weak **1°; 2°** craspedodromous with irregular spacing and two pair acute basal secondaries angle; **3°** alternate percurrent and angle with respect to **1°** obtuse; **4°** regular polygonal reticulate; **Marginal ultimate venation** looped; **Areoles** moderately developed.	Blade class notophyll
Cucumis anguria var ***longaculeatus*** (including 2 accessions of ***C. anguria***- GB-00062 and GB 000554	**Lamina** orbiculate with convex apex, odd-lobed obtuse apex angle, lobate base, wide-obtuse base angle and spinose tooth apex, symmetrical, mesophyll, serrated, palmately lobed. **Venation** actinodromous suprabasal, straight branched, weak **1°; 2°** craspedodromous with irregular spacing and two pair acute basal secondaries angle; **3°** alternate percurrent and angle with respect to **1°** obtuse; **4°** regular polygonal reticulate; **Marginal ultimate venation** looped; **Areoles** moderately developed.	Blade class mesophyll
Cucumis metuliferus	**Lamina** orbiculate with convex apex, odd-lobed acute to odd-lobed obtuse apex angle, lobate base, wide-obtuse base angle and spinose tooth apex, symmetrical, notophyll to mesophyll, serrated, palmately lobed. Venation actinodromous suprabasal, straight branched, weak 1°; 2° craspedodromous with irregular spacing and two pair acute basal secondaries angle; 3° alternate percurrent and angle with respect to 1° acute; 4° regular polygonal reticulate; Marginal ultimate venation looped; Areoles moderately developed.	3° vein angle with respect to 1° alternate percurrent

Figure 1 shows the result of cluster analysis. It was observed that species and accessions of *Cucumis* were delineated based on leaf architecture characters. Distinguishing features examined successfully separates species and accessions into different clusters.

DISCUSSION

Species and accessions of *Cucumis* primarily differed in secondary vein spacing separating *C. melo* from other four species (*C. metuliferus*, *C. anguria*, *C. anguria* var *longaculeatus* and *C. myriocarpus*). In advance dicots and monocots, one of the striking features of vascular pattern was spatial regularity of leaf veins. Despite of the differences in leaf shape, regularity in venation patterns was also apparent in the reticulate venation of dicots. Considering the regularity of this leaf vein character, it is considered as a strong character in delineating taxonomic groups. Differences could be seen in the uniformity of the veins which could either be primary, secondary, tertiary or higher vein orders (Nelson and Dengler, 1997). Venation patterns are indeed significant features for the classification and even tracing the evolution of angiosperms since these are relatively stable at the species level and noted to be genetically fixed which further support the importance of these characters in taxonomy and systematics (Roth-Nebelsick *et al*., 2001). Thus, venation pattern is a distinguishing feature of *Cucumis* species and accessions.

Variations in the primary vein size separated accessions of *C. melo*. Primary vein size and leaf size were associated to each other. Accessions with weak primary vein size were observed to have small leaf size. Three accessions with moderate primary vein size had larger leaves compared to other accession. Primary vein characteristics were used to described and classify morphologically similar leaves of some species like *Podocarpus* (Salvaña *et al*., 2018) and subsections of Anthurium (Mantovani *et al*., 2009).

Accessions of *C. metuliferus* were divided into two groups based on apex angle. This character and others that require measurement are part of numerical approaches used in describing species. Qualitative characters like apex angle are dependent on these numerical values since these represent corresponding qualitative categories (Hill, 1980). Further analysis can be made in order to provide proof on identification and classification based on these characters. Apex angle is also related to leaf shape which is the basic recognized leaf character. Measurements are more or less definite, thus, classifying and identifying species based on apex angle are reliable (Nandyal *et al*., 2013).

Blade class was the distinguishing feature of *C. anguria* and *C. anguria* var *longaculeatus* in which two accessions (GB-000062; GB-000554) of *C. anguria* had similar blade class to *C. anguria* var longaculeatus. Blade class is commonly categorized based on the surface area of the leaf. Accessions classified with mesophyll leaves have larger surface area compared to accessions with notophyllous leaves. Blade classes of Cucumis, as a high-value crop, can be used as a commercial tool in identifying different accessions, especially in breeding programs, since this character was used in the classification of accessions like in Glycine max (Chen and Nelson, 2004). Controversial and problematic species in a specific genus and infraspecific taxa were successfully split and classified based on blade class (Baroga and Buot, 2014; Kpadehyea and Buot, 2014; Borazan and Babac, 2003). This character was also used in clarifying controversial lumping of species (Laraño and Buot, 2010). Based on these arguments and leaf architectural character, the two accessions of *C. anguria*

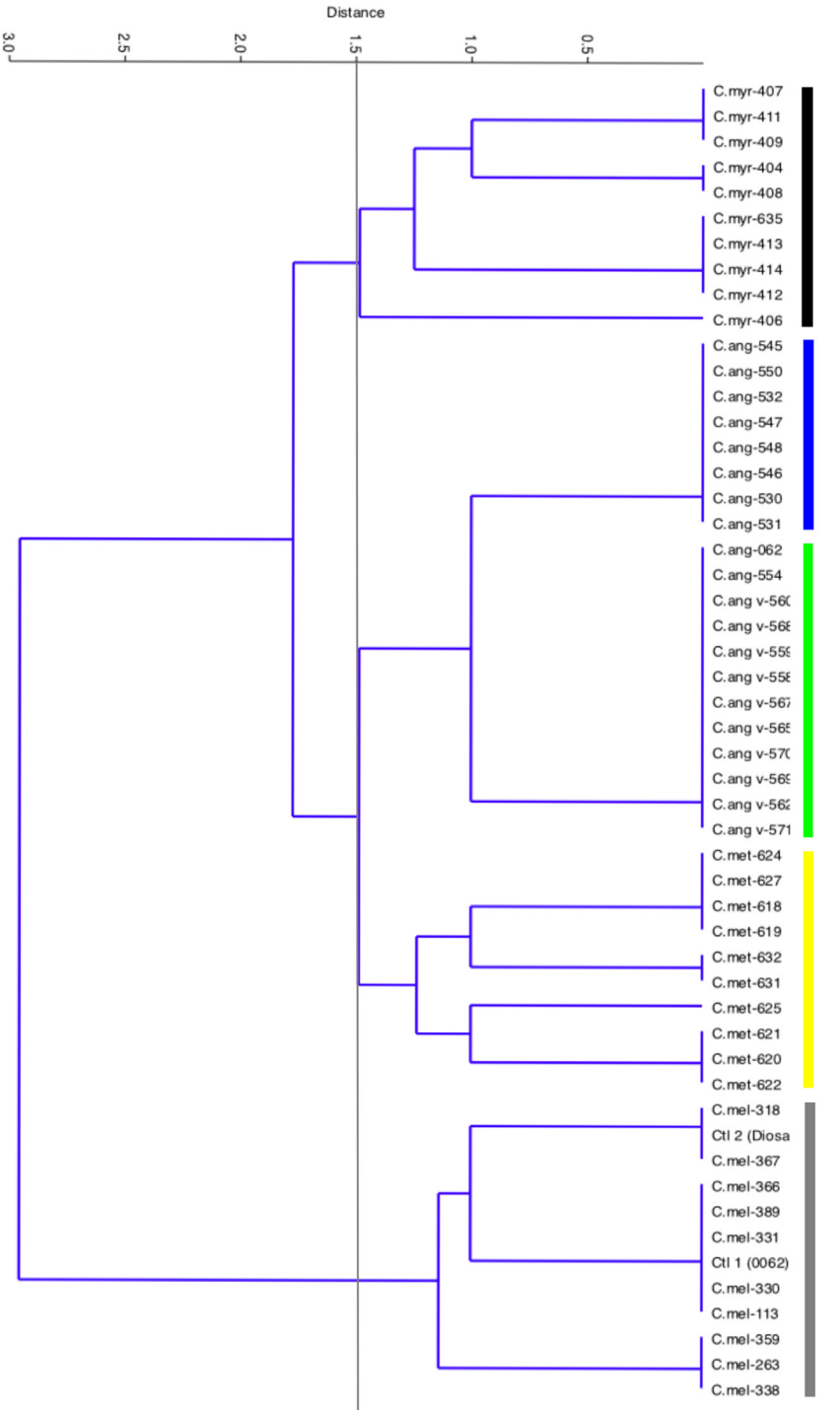

Figure 1. Unweighted Pair Group Method using Averages (UPGMA) dendrogram, based on Euclidean distance coefficient (1.5), of different accessions of different species of *Cucumis*: (■) *C. myriocarpus*; (■) *C. anguria*; (■) *C. anguria* var *longaculeatus*, including two accessions of *C. anguria* (GB-000062 and GB-000554); (■) *C. metuliferus* ; and (■) *C. melo.*

belong to the group of *C. anguria* var *longaculeatus* and could possibly be accessions of the latter species.

One of the fundamental character used to describe a particular species is laminar shape (Hickey, 1973). This character separates one accession of *C. myriocarpus* from other accessions. Since variations may exist as it can be affected by environmental variables, a qualitative range of laminar shape can be used. Nevertheless, laminar shape is commonly used in identifying unknown species. Variabilities of laminar shape were commonly observed among species of some genera which were used to classify these species (Bhat, 1995). Laminar shape can also be associated to leaf surface area since it is determined by getting the length and width ratio of the lamina. It is one of the most easily recognized character in comparing and delineating interspecific and intraspecific taxa (Kpadehyea and Buot, 2014).

The results of this study provide additional importance and utilities of leaf architecture in the identification and classification of species and accessions, particularly cultivated species like *Cucumis*. Variations examined in this study provide good insights in the identification and classification of each accessions of *Cucumis* species for better management of these accessions. It also supported the placement of each accessions in each species as supported by the result of the cluster analysis.

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