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Resolving Taxonomic Confusion through Leaf Architecture: The Case of Genus *Cucumis* L.

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| **Received:** 26 February 2020 | **Accepted:** 25 April 2020 |

How to cite: Averion-Masungsong L, Buot, Jr. IE. 2020 Resolving Taxonomic Confusion through Leaf Architecture: The Case of Genus *Cucumis* L. J New Biol Rep 9(1): 86 – 93.

ABSTRACT

The presence of morphological resemblances of *Cucumis* L. species is the main reason of taxonomic confusion within this group. This study aims to confirm the identity of *Cucumis* species through characterization and identification of leaf architecture patterns. Five *Cucumis* species were considered namely, *Cucumis melo*, *Cucumis anguria*, *Cucumis anguria* var. *longaculeatus*, *Cucumis myriocarpus*, and *Cucumis metuliferus*. Leaf samples were collected at the East West Seed Company Screen house, Lipa City, Batangas, Philippines. 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. Dichotomous key was constructed based mainly on the observed leaf architectural features. Unifying characters of *Cucumis* species include laminar symmetry, primary vein, secondary vein, quaternary vein, secondary vein angle, apex shape, base shape, base angle, margin, tooth shape and tooth apex. On the other hand, each *Cucumis* species were distinguished based on blade class, laminar shape, secondary vein spacing, tertiary vein category, apex angle, lobation and areole development. In terms of leaf venation, *Cucumis* species can be distinguished based on secondary vein spacing. Results showed that leaf architectural characters are of great help in the identification and classification of *Cucumis* species.

Key words: Leaf architecture, description, *Cucumis* species, morphology.

INTRODUCTION

The genus *Cucumis* L. (Cucurbitaceae) is composed of about 30 wild and cultivated species which have originated from Africa (Bisognin 2002). Variations among these species have been recognized which makes identification and classification more difficult. There is a need to reconcile and validate the existing classification of

the *Cucumis* species used in this study and leaf architecture will be of great help.

Leaf architecture is one of the most important morpho-anatomical tools which is highly useful in species delineation. Leaves, despite phenotypic plasticity, have genetically-fixed and established venation patterns which are significant when dealing with sterile fossil plants and rarely flowering species (Roth-Nebelsick et al. 2001,

Salvaña et al. 2019). Several studies utilize various leaf characters in identifying and classifying plant taxa. One is that of Laraño & Buot (2010) which supported the merging of families Malvaceae, Sterculiaceae, Tiliaceae and Bombacaceae into family Malvaceae as described in the Angiosperm Phylogeny Group (APG). Other studies with significant results were that of *Psychotria* species (Banaticla & Buot 2010), Philippine *Cinnamomum* species (Celadiña et al. 2012), *Terminalia* species (Baroga & Buot 2014), and *Hoya* species (Salvaña & Buot 2014, Jumawan & Buot 2016). Most of them give invaluable results proving the importance of leaf architecture in morphology as well as in taxonomy of controversial taxa.

Leaf architecture can significantly contribute to the proper identification and classification of *Cucumis* species. This present study entails the complete leaf architectural study of *Cucumis* species which can be beneficial to plant repositories in the re-establishment and/or re-arrangement of various standing collections of *Cucumis*. Results can be also used as a key guide in the identification of wild species.

MATERIALS AND METHODS

Leaf samples from five systematically planted *Cucumis* species were examined namely: (1) *Cucumis anguria*; (2) *C. anguria* var. *longaculeatus*; (3) *C. melo*; (4) *C. metuliferus*; and (5) *C. myriocarpus*. Ten leaf samples per species were chosen. Three fully expanded leaves per sample were collected at the Hortanova Research Center, East West Seed Company Inc. screen house, Lipa City, Batangas, Philippines. 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. Representative leaf samples were sketched for the venation characters. Twelve general leaf characters and eight vein characters were used in examining the collected leaf samples. Measurements were done using a ruler, a caliper, and a protractor. Dichotomous key was constructed based mainly on the observed leaf architectural features.

RESULTS

Leaf Architecture Patterns of *Cucumis* L.

The *Cucumis* species examined in this study have the following unifying characters: laminar symmetry, primary vein, secondary vein, quaternary vein, secondary vein angle, apex shape, base shape, base angle, margin, tooth shape and tooth apex. The most evident delineating features of the five species are the blade class, laminar shape, secondary vein spacing, tertiary vein category, apex angle, lobation and areole development. In addition, *Cucumis* leaves were observed to have serrated margin, symmetrical lamina and convex apex. Its base shape generally lobate with wide obtuse base angle, tooth apex spinose and with concave apical and basal tooth sides.

Among the twelve (12) general leaf characters examined four characters namely: blade class, laminar shape, apex angle, and lobation can separate the five species (Table 1).

Table 1. Leaf architecture patterns of *Cucumis* species.

LEAF CHARACTERS	SPECIES				
	<i>C. melo</i>	<i>C. myriocarpus</i>	<i>C. anguria</i>	<i>C. anguria</i> var. <i>longaculeatus</i>	<i>C. metuliferus</i>
Blade Class	mesophyll	notophyll to mesophyll	notophyll to mesophyll	mesophyll	notophyll to mesophyll
Laminar Symmetry	symmetrical	symmetrical sub-orbiculate	symmetrical	symmetrical	symmetrical
Laminar Shape	orbiculate	to orbiculate	orbiculate	orbiculate	orbiculate
Range L:W Ratio	0.92:1 – 1.06:1	0.96:1 – 1.29:1	0.90:1 – 1.03:1	0.92:1 – 1.09:1	0.92:1 – 1.07:1
Apex Angle	obtuse	odd-lobed obtuse	odd-lobed obtuse	odd-lobed obtuse	odd-lobed obtuse
Apex Shape	convex	convex	convex	convex	convex
Base Angle	wide obtuse	wide obtuse	wide obtuse	wide obtuse	wide obtuse
Base Shape	lobate	lobate	lobate	lobate	lobate
Margin	serrate	serrate	serrate	serrate	serrate
Tooth Apex	spinose	spinose	spinose	spinose	spinose
Tooth Shape (Apical/Basal)	concave/ concave	concave/ concave	concave/ concave	concave/ concave	concave/ concave
Lobation	unlobed to palmately lobed	palmately lobed	palmately lobed	palmately lobed	palmately lobed

Cucumis melo and *Cucumis anguria* var. *longaculeatus* exhibited mesophyll blade (5,000 - 14,000 mm² leaf area) while *Cucumis metuliferus*, *Cucumis myriocarpus* and *Cucumis anguria* have notophyll to mesophyll blade (1,500 – 8,000 mm² leaf area). Generally, *Cucumis* leaves have elliptic lamina, wherein the axis of greatest width is perpendicular to the approximate midpoint of the leaf axis. Of the five species studied four have orbiculate type of elliptic lamina (L:W Ratio of 1:1), delineating *Cucumis myriocarpus* species with sub-orbiculate type (L:W Ratio of 1.2:1). In terms of apex angle, *C. myriocarpus*, *C. anguria*, *C. anguria* var. *longaculeatus* and *C. metuliferus* have an odd-lobed obtuse type while *C. melo* have an obtuse type. This also goes in terms of lobation, same four species were observed to be palmately-lobed while *C. melo* exhibited unlobed to palmately lobed type.

Cucumis species can be primarily separated based on secondary vein spacing. The secondary vein spacing of *C. myriocarpus*, *C. anguria*, *C. anguria* var. *longaculeatus* and *C. metuliferus* was observed to be irregular while *C. melo* was increasing towards the base. On the basis of tertiary vein angle to primary, all the species exhibited an obtuse type except *C. metuliferus* which have an acute tertiary vein angle to primary. Another delineating feature is the tertiary vein angle were three species have alternate percurrent type and the two species have random reticulate type. Likewise, areole development can differentiate the studied species. Three species have moderately developed areoles while *C. myriocarpus* had moderately to well-developed areoles and *C. melo* had well-developed areoles. All examined species have actinodromous suprabasal primary veins, craspedodromous secondary veins, two-pair acute basal secondaries secondary vein angles, and regular polygonal reticulate quaternary vein (Table 2).

Leaf Vein Characters of *Cucumis* L.

Table 2. Vein characteristics of the *Cucumis* leaves.

VEIN CHARACTERS	SPECIES				
	<i>C. melo</i>	<i>C. myriocarpus</i>	<i>C. anguria</i>	<i>C. anguria</i> var. <i>longaculeatus</i>	<i>C. metuliferus</i>
1° vein	actinodromous suprabasal	actinodromous suprabasal	actinodromous suprabasal	actinodromous suprabasal	actinodromous suprabasal
2° vein	craspedodromous	craspedodromous	craspedodromous	craspedodromous	craspedodromous
2° vein spacing	increasing towards the base	irregular	irregular	irregular	irregular
2° vein angle	two pair acute basal secondaries	two pair acute basal secondaries	two pair acute basal secondaries	two pair acute basal secondaries	two pair acute basal secondaries
3° vein angle to 1°	obtuse	obtuse	obtuse	obtuse	acute
3° vein	alternate percurrent	random reticulate to alternate percurrent	random reticulate to alternate percurrent	alternate percurrent	alternate percurrent
4° vein	regular polygonal reticulate	regular polygonal reticulate	regular polygonal reticulate	regular polygonal reticulate	regular polygonal reticulate
Areole development	well-developed	moderately to well-developed	moderately developed	moderately developed	moderately developed

Key to Five Species of *Cucumis* L. Based on Leaf Architecture

1. Leaf unlobed to palmately lobed, apex obtuse and 2° vein spacing increases towards the base.....*Cucumis melo*
1. Leaf palmately lobed, apex odd-lobed obtuse and 2° vein spacing irregular.....2
2. Lamina sub-orbiculate and with moderately to well-developed areoles.....*Cucumis myriocarpus*
2. Lamina orbiculate and with moderately developed areoles..... 3
3. Leaf blade 72 to 145 mm long, mesophyll blade class.....*Cucumis anguria* var. *longaculeatus*

- 3. Leaf blade 33 to 114 mm long, notophyll to mesophyll blade class.....4
- 4. 3° vein alternate percurrent with acute 3° vein angle to 1°.....*Cucumis metuliferus*
- 4. 3° vein random reticulate with obtuse 3° vein angle to 1°.....*Cucumis anguria*

***Cucumis melo* L.**

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.

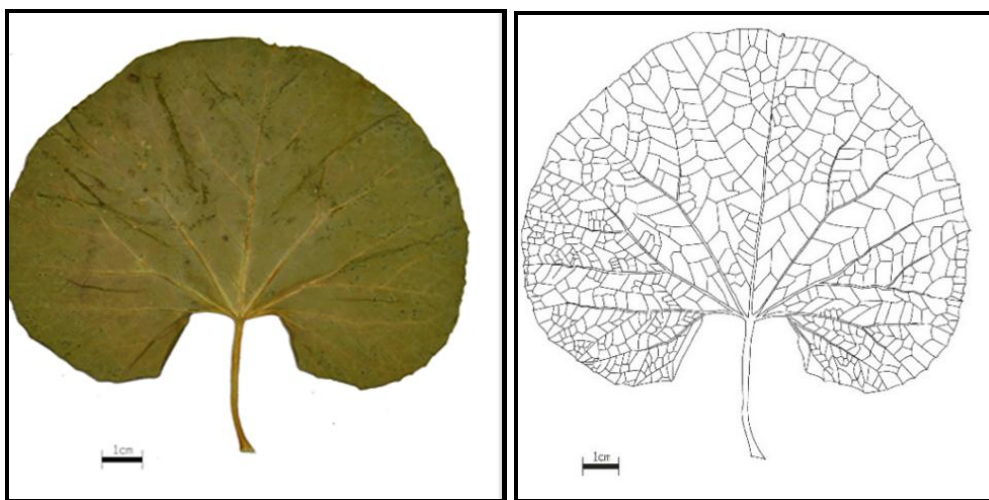


Fig. 1. Leaf sample and venation pattern of *C. melo*.

***Cucumis myriocarpus* E. Mey ex Naud**

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** looped; **Areoles** moderately to well-developed.

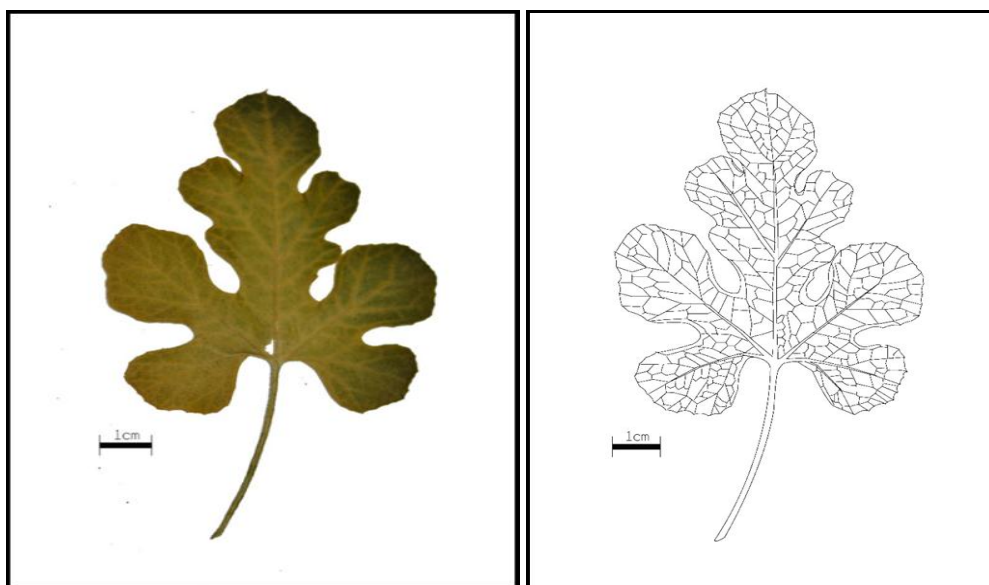


Fig 2. Leaf sample and venation pattern of *C. myriocarpus*.

Cucumis anguria var. *longaculeatus* J.H. Kirkbride

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.

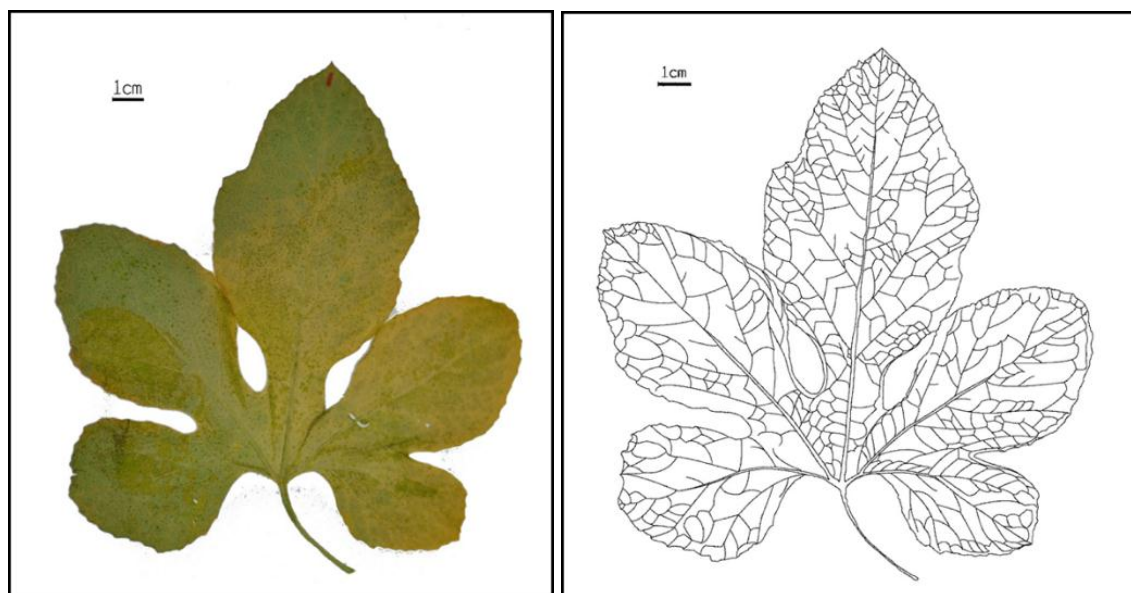


Fig. 3. Leaf sample and venation pattern of *C. anguria* var. *longaculeatus*.

Cucumis metuliferus E. Mey

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.

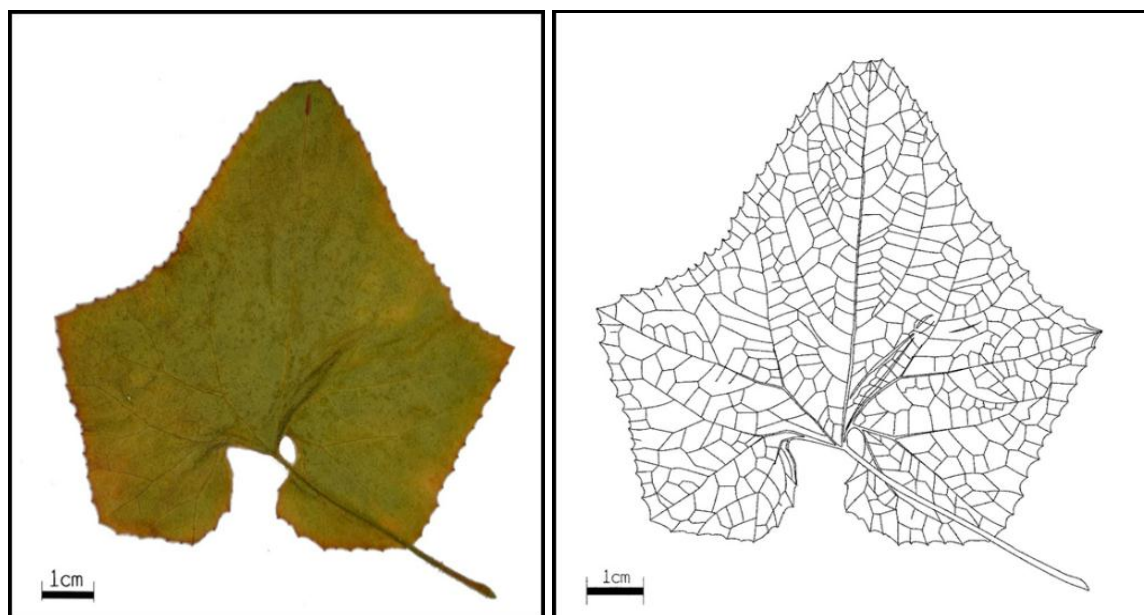


Fig. 4. Leaf sample and venation pattern of *C. metuliferus*.

***Cucumis anguria* L.**

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.

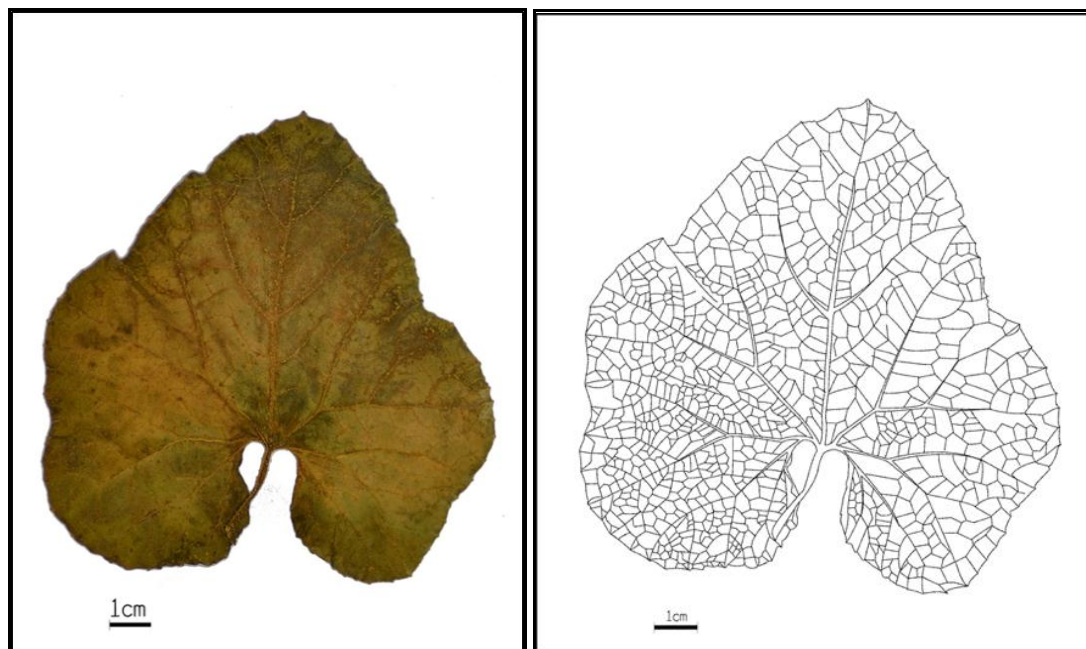


Fig. 5. Leaf sample and venation pattern of *C. anguria*.

DISCUSSION

Cucumis species were distinguished based on blade class, laminar shape, secondary vein spacing, apex angle, lobation and areole development. Blade class was also used in splitting and separating species in a specific genus and infraspecific taxa (Baroga & Buot 2014). Lamina shape can be associated to leaf surface area since it was determined by getting the length and width ratio of the lamina. One of the morphological character used in identifying plant species was the shape of the lamina. It was one of the most easily recognized character in comparing and delineating interspecific and intraspecific taxa (Kpadehyea & Buot 2014). Apex angle together with other measurements are part of numerical taxonomic approaches used to describe species. This provides numerical values which has corresponding qualitative character. Measurements are more or less definite, thus, classifying and identifying species based on apex angle are reliable (Hill 1980, Nandyal et al. 2013). Also, leaf lobes were noted to be essential to discriminate specific vein patterns and can display accurate homological and/or ancestral relationships (Viscosi & Cardini 2011). Lobation and primary vein size were also some of the leaf characters served as basis for classifying the bilobate leaf fossils and evaluating the fossil record and biogeography of *Bauhinia* species (Lin et al. 2015). Lobation can be associated with the leaf surface area. Palmately

lobed species have smaller surface area compared to unlobed species.

In terms of venation patterns, they had craspedodromous 2° veins, alternate percurrent 3° veins and regular polygonal reticulate 4° veins. They had actinodromous suprabasal 1° veins, and looped marginal ultimate venation. In the leaf architectural study conducted by Rao & Rao (2015) in some members of family Cucurbitaceae, they have also observed some of these leaf characters in the representative species of *Cucumis*.

Variations in leaf venation patterns are valuable in identifying taxonomic group, differentiating species and even delineation of different accessions and/or varieties (Badron et al. 2014). All of the fifty *Cucumis* accessions studied have reached at least 4° vein order which according to Sack & Scoffoni (2013), should have achieved maximum rate of water transport, which can be correlated to maximum rate of photosynthesis achieved. In addition, they can also provide cost-efficiency for biomechanical support and protection against damage cause by insects and herbivores. Furthermore, *C. melo* differ from other four species through secondary vein spacing. Secondary vein spacing is related to lobation. It was observed that accessions with irregular secondary vein spacing were palmately lobed while those that have secondary vein spacing increasing towards the base were unlobed. Secondary veins were noted to act as support system of the lamina. Nelson & Dengler

(1997) added that one of the striking features of vascular pattern is spatial regularity of leaf veins especially in advanced dicots and monocots. This regularity is also apparent in the reticulate venation of dicots despite of the differences in leaf shape. Considering the regularity of this leaf vein character, it is considered as a strong character in delineating taxonomic groups. Differences can be seen in the uniformity of the veins which can either be primary, secondary, tertiary or higher vein orders.

Variability of areole development, including size and shape, are taxonomically important since they provide useful information as a distinguishing feature of closely related taxa or groups (Bhat 1995). Areole morphology was also important in the study of primitive taxa in comparison with the advanced group (Boke 1957). In addition, Sack & Scoffoni (2013) stated that high number of areoles per unit leaf area can correlate with, and may provide the advantages of, high VLA. This means that well-developed areolation in relation to high vein length per unit area can provide tolerance of fine-scale damage to the leaf, enable higher leaf conductance, greater stomatal density and stomatal conductance, and higher rates of gas exchange per unit leaf area. They may also provide a possible advantage against insect herbivory. Lamina shape and areole development were some of the leaf characters used by Loufty et al. (2005) to show similarities and differences of some species of the genus *Ficus* L.

CONCLUSION

The leaves of the *Cucumis* species studied show close similarities with each other. But upon examination of its leaf characters, it can be delineated from one another. Blade class, lamina shape, apex angle and lobation are important species markers. This study showed the importance of leaf architecture specially in delineating species with confusing taxonomic status like *Cucumis* L.

ACKNOWLEDGEMENTS

The author would like to thank Department of Agriculture - Bureau of Agricultural Research and Department of Science and Technology for financing this study and Hortanova Research Center, East West Seed Company Inc., for providing the *Cucumis* leaves used.

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